THE

AMERICAN JOURNAL

OF THE MEDICAL SCIENCES

MARCH, 1938

ORIGINAL ARTICLES.

THE INFLUENCE OF MUCIN UPON THE ABSORPTION OF IRON IN HYPOCHROMIC ANEMIA.

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Mucus has a number of obvious qualities which can act to protect surface membranes of the body: mucus binds acids, holds foreign bodies (bacteria), can absorb an enormous amount of water, and holds enzymes of the stomach and intestine (Boldyreff[1]). Boldyreff believed that if present in large quantities mucus can seriously hinder digestion, weakening the action of digestive ferments and slowing down absorption of digested food. Davies,[2] among others, has called attention to the increase of mucus in the gastric contents of patients with chronic hypochromic anemia and achlorhydria. It has often been assumed for various reasons that the absorption of iron from the intestine of these patients is defective. Although the presence of mucus has not been considered a

factor in the malabsorption of iron, the possibility is apparent. The following observations were made to ascertain whether or not iron fed to patients with hypochromic anemia in the presence of a large amount of mucin would be utilized to a less extent than iron given alone.

Method. Ten cases of hypochromic anemia, or iron deficiency, were studied (see Table 1). Although blood loss was probably the principle cause of iron deficiency in all patients, blood loss had ceased before the periods of observation began. The patients were given a basal diet which was rather low in iron. A summary of the hemoglobin and reticulocyte percentages and the amount of iron and mucin administered is recorded in Table 2. Reticulocytes were counted daily upon smears of capillary blood stained supravitally with brilliant cresyl blue and counterstained with Wright's stain. Venous blood was obtained every other day, and prevented from clotting by oxalate. Upon this blood the red blood cell count, hemoglobin percentage, hematocrit (cell volume), mean corpuscular volume and mean corpuscular hemoglobin concentration were determined, according to the method of Wintrobe. The hemoglobin percentage was determined with the Sahli apparatus calibrated so that 100% hemoglobin was equivalent to 15.6 gm. per 100 cc. or 20.9 volumes % oxygen capacity (Van Slyke).

"Gastric Mucin"* was prepared in suspension of 9 to 15%. The ferrous sulphate was given in 5% glucose solution, excepting when over 0.1 gm. three times daily was given, when it was administered in tablet form.

Results. In all cases in which mucin and iron were administered together (Cases 1, 2, 3, 4, 5), there was evidence that the mucin inhibited the utilization of iron. The criteria on which this conclusion is based are as follows: when a given amount of iron was mixed with mucin and administered daily for about 10 days and when the same amount of iron without mucin was then given daily during

TABLE 1.—SUMMARY OF CASES.

Case No.	Age.	Sex.	Diagnosis and etiologic factors.
1	68	M	Hypochromic anemia of blood loss from duodenal ulcer. Normal gastric acidity.
2	14	F	Hypochromic anemia of blood loss from gastric ulcer. "Chlorosis." Normal gastric acidity.
3	37	F	Hypochromic anemia of blood loss from duodenal ulcer.
4	51	F	Hypochromic anemia of blood loss from duodenal ulcer. Asymptomatic tertiary syphilis. Uterine fibroid (bleeding). Normal gastric acidity.
5	45	F	Hypochromic anemia of blood loss from duodenal ulcer.
6	67	F	"Idiopathic" hypochromic anemia. No history of blood loss. Poor diet. Arteriosclerotic heart disease. Gastric anacidity after the injection of histamine.
7	52	F	"Idiopathic" hypochromic anemia. Menorrhagia. Dysphagia. Gastric anacidity after the injection of histamine.
8	22	M	Hypochromic anemia of blood loss from gastric ulcer. Normal gastric acidity.
9	64	F	"Idiopathic" hypochromic anemia. No history of blood loss. Bronchopneumonia, healing. Gastric anacidity after the injection of histamine.
10	57	M	Hypochromic anemia of blood loss from hemorrhoids. Gastric anacidity after the injection of histamine.

(NOTE: Bleeding ceased in all cases before the periods of observation began.)

* Obtained from Frederick Stearns & Co., Detroit.

TABLE 2.—SYNOPSIS OF HEMOGLOBIN AND RETICULOCYTE PERCENTAGES AND MODIFICATIONS OF IRON THERAPY IN 10 CASES OF HYPOCHROMIC ANEMIA.

TABLE 2.—SYNOPSIS OF HEMOGLOBIN AND RETICULOCYTE PERCENTAGES AND MODIFICATIONS OF IRON THERAPY IN 10 CASES OF HYPOCHROMIC ANEMIA.

Consecutive periods.	Days.	Case 1. Hgb.	Case 1. Retics.	Case 2. Hgb.	Case 2. Retics.	Case 3. Hgb.	Case 3. Retics.	Case 4. Hgb.	Case 4. Retics.	Case 5. Hgb.	Case 5. Retics.	Case 6. Hgb.	Case 6. Retics.	Case 7. Hgb.	Case 7. Retics.	Case 8. Hgb.	Case 8. Retics.	Case 9. Hgb.	Case 9. Retics.	Case 10. Hgb.	Case 10. Retics.
Control	0	42	5.9	50	1.0	49	11.7	51	0.7	54	4.2	38	4.2	32	1.1	44	...	65	1.5	36	0.5
	2	43	3.6	54	5.9	52	6.3	51	1.2	59	3.3	40	2.6	32	0.7	47	2.5	65	1.1	35	1.8
	4	47	2.9	53	5.5	53	2.6	51	1.1	58	4.3	41	3.7	32	0.8	50	0.7	65	1.4	34	1.5
	6	47	2.8	59	3.9	58	3.9	..	...	57	2.7	..	...	32	1.0	47	0.7	64	1.1		
	8	45	2.2	57	2.2	60	6.3	..	...	59	2.1	..	...	33	0.9	46	0.5	63	1.3		
	10	42	1.4	56	1.6	59	3.6	..	...	58	2.1	..	...	33	0.6	43	0.5	63	1.7		
	12	..	...	54	1.0	61	2.2	..	...	59	1.8	..	...	33	0.8	41	0.7	62	0.8		
	14	..	...	54	1.0	64	1.9	..	...	56	2.2	..	...	33	0.9	42	0.4	66	0.6		
	16	..	...	..	...	64	2.2	..	...	58	2.0	..	...	33	1.8						
		Iron and ammonium citrate, 0.3 gm., mixed with mucin, 1.2 gm. t.i.d.		Iron and ammonium citrate, 0.3 gm., mixed with mucin, 1.2 gm. t.i.d.		Ferrous sulphate, 0.065 gm., mixed with mucin, 1.8 gm. t.i.d.		Ferrous sulphate, 0.062 gm., mixed with mucin, 1.7 gm. t.i.d.		Ferrous sulphate, 0.084 gm., mixed with mucin, 1.5 gm. t.i.d		Ferrous sulphate, 0.08 gm., given immediately after mucin, 4.5 gm. t.i.d.		Ferrous sulphate, 0.09 gm., given immediately after mucin, 4.5 gm. t.i.d.		Ferrous sulphate, 0.10 gm., given 1 hour after mucin, 1.7 gm. t.i.d.		Iron and ammonium citrate, 0.28 gm., mixed with 150 cc. 40% cream, t.i.d.		Ferrous sulphate, 0.08 gm., mixed with 150 cc. 40% cream, t.i.d.	
First observation	2	39	2.2	55	2.7	63	**3.8**	47	0.9	60	2.3	41	5.1	33	3.7	44	3.6	62	0.9	34	3.8
	4	43	2.4	58	**6.5**	64	3.3	51	**1.9**	59	2.0	41	**11.7**	36	**5.7**	45	3.9	62	2.0	38	**6.3**
	6	46	**3.1**	61	6.2	60	3.7	50	1.1	59	1.7	42	8.7	35	4.2	50	7.4	64	**3.5**	42	5.3
	8	48	2.3	62	4.0	63	3.6	51	1.3	60	**3.2**	43	8.1	36	2.7	50	10.1	66	2.4	42	3.4
	10	49	2.3	64	5.7	64	2.7	52	0.8	62	1.7	45	8.1	38	3.8	51	**11.3**	65	1.6	40	2.6
	12	..	...	..	...	65	2.7	53	1.2	63	1.5	45	3.4	41	4.1	56	6.1	64	1.6		
	14	..	...	..	...	..	...	..	...	..	...	46	3.0	..	...	60	5.3				
		Iron and ammonium citrate, 0.3 gm. t.i.d.		Iron and ammonium citrate, 0.3 gm. t.i.d.		Ferrous sulphate, 0.045 gm. t.i.d.		Ferrous sulphate, 0.05 gm. t.i.d.		Ferrous sulphate, 0.09 gm. t.i.d.		Ferrous sulphate, 0.09 gm. t.i.d.		Ferrous sulphate, 0.09 gm. t.i.d.		Ferrous sulphate, 0.10 gm. t.i.d.		Iron and ammonium citrate, 0.28 gm. t.i.d.		Ferrous sulphate, 0.08 gm. t.i.d.	
Second observation	2	50	3.8	66	6.7	66	2.2	55	**3.2**	64	1.6	46	2.2	42	1.9	60	5.5	65	2.1	42	2.8
	4	53	**4.3**	70	**8.1**	67	2.0	59	3.2	65	2.7	47	5.8	44	1.5	60	6.4	65	0.8	45	2.4
	6	58	3.2	76	6.1	70	1.9	58	1.8	65	2.0	48	**10.0**	47	1.5	61	4.6	65	1.8	45	2.2
	8	62	3.0	74	4.6	75	1.5	59	1.8	70	**2.9**	55	8.6	47	1.1	63	2.1	67	1.8	44	3.6
	10	62	2.9	80	2.8	78	1.1	60	1.1	..	...	52	3.5	48	0.7	66	1.9	68	0.9	44	3.5
	12	64	2.3	..	...	78	1.4	60	0.9	..	...	60	3.2	51	1.2	71	1.4	67	0.9	47	1.1
	14	61	0.8	..	...	..	...	59	1.3	..	...	60	2.3	54	2.5	74	1.2	..	...	47	1.6
	16	64	1.4	..	...	..	...	..	...	..	...	60	1.4	59	2.0	..	...	..	...	47	3.0
	18	66	2.8	..	...	..	...	..	...	..	...	60	2.1							48	3.6
	20																				
						Ferrous sulphate, 0.25 gm. t.i.d.		Ferrous sulphate, 0.25 gm. t.i.d.								Ferrous sulphate, 0.25 gm. t.i.d.		Ferrous sulphate, 0.25 gm. t.i.d.			
Third observation	2	..	...	..	...	78	1.6	57	3.2	..	...	..	...	..	...	77	1.4	64	1.3		
	4	..	...	..	...	80	1.3	59	3.2	..	...	..	...	..	...	78	0.9	64	0.7		
	6	..	...	..	...	80	1.4	63	**5.1**	..	...	..	...	..	...	76	...	66	0.5		
	8	..	...	..	...	..	...	68	4.5	..	...	..	...	..	...	80	...	70	0.3		
	10	..	...	..	...	..	...	65	3.3												
	12	..	...	..	...	..	...	65	3.2												

a second consecutive period of about 10 days either a more rapid hemoglobin increase, a second reticulocyte response, or both followed the administration of iron without mucin. A principle is thus employed which has been used to test the therapeutic effectiveness of hemopoietic substances. Minot and Castle[4] have described the method in detail. In Case 2, for example, iron and ammonium citrate, 0.3 gm., mixed with mucin, 1.2 gm., was administered 3

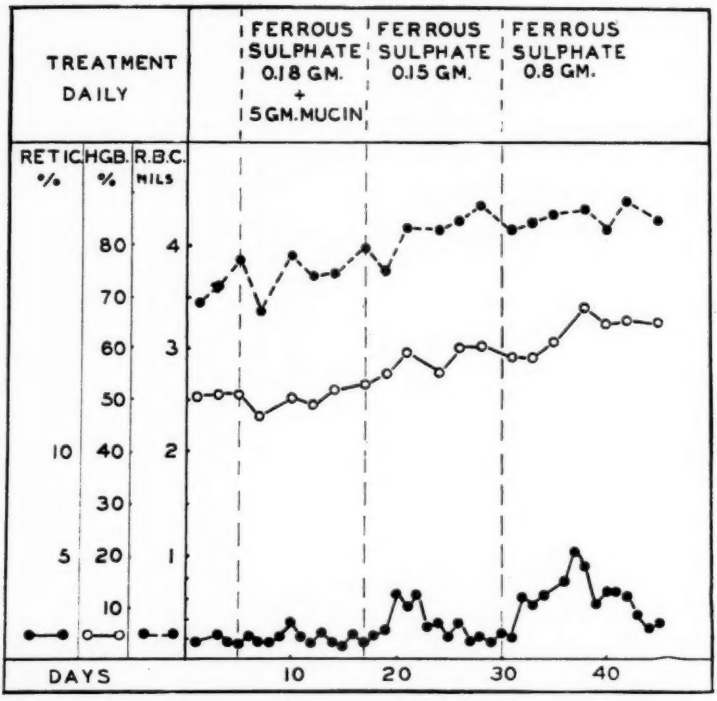

FIG. 1.—Case 4 (Tables 1 and 2). The inhibiting influence of mucin upon the utilization of iron as shown chiefly by the comparative reticulocyte responses. A negligible reticulocyte response followed the administration of ferrous sulphate mixed with mucin; a definite reticulocyte response followed the omission of mucin; an even greater reticulocyte response followed the administration of an optimal dose of ferrous sulphate.

times daily. There followed a hemoglobin increase of about 8%. A reticulocyte response occurred with a peak of 6.5% on the fourth day. On the tenth day the mucin was stopped and treatment with iron and ammonium citrate, 0.3 gm., 3 times daily was continued. During this second period the hemoglobin increased a little more rapidly (about 10% in 8 days) than during the first period, and there was a second reticulocyte response with a peak of 8.1% on the

fourth day. The important point is that a second reticulocyte response occurred and not that its magnitude was greater than the first. A second response when therapy is daily and continuous implies that the material given in the second period is more potent than in the first period. The conclusion may be drawn, therefore, that the iron medication was more effective in the second period than in the first, and that the mucin, which was the only variant, inhibited in some manner the influence of the iron medication in the first period. This conclusion seems warranted also in Cases 1, 3, 4, and 5. In Case 3, no second reticulocyte response occurred, but no definite hemoglobin increase followed the administration of ferrous sulphate mixed with mucin, whereas a very definite hemoglobin increase followed the administration of ferrous sulphate alone. One must be cautious in comparing hemoglobin rises in two equal consecutive periods during iron administration. Since the hemoglobin does not usually begin to increase until several days after iron therapy has commenced, the total hemoglobin rise during the first period may not be as great as during the second period, when the effectiveness of the iron may be the same in each period. Furthermore, there tends to be a slower rate of hemoglobin manufacture as the hemoglobin level rises.

The doses of iron given were purposely small, and much less than the optimal amount to be used in the routine treatment of chronic hypochromic anemia. It is likely that if large doses of iron had been given, no inhibiting effect of mucin would have been demonstrable. In a third observation in Case 4 (Fig. 1) an optimal dose of ferrous sulphate was followed by a third reticulocyte peak, even higher than the preceding two. This merely indicates that ferrous sulphate 0.25 gm., given 3 times daily, was more effective than one-fifth this dose, which was suboptimal in this particular case.

When ferrous sulphate was given to patients separate from, but immediately after, mucin the effect was variable. In Case 6 there was a second reticulocyte response and a faster hemoglobin increase when ferrous sulphate was given without mucin, indicating that in the first period the mucin had inhibited the utilization of iron. In Case 7, there was no second reticulocyte response and no very definite increased formation of hemoglobin, so that there was no evidence that the mucin inhibited the utilization of iron in this particular case. In Case 8, ferrous sulphate was administered one hour after mucin. No increased hemoglobin formation and no second reticulocyte response occurred. One additional case (not recorded in Table 2) was given ferrous sulphate ½ hour after mucin, and then after 10 days ferrous sulphate alone was administered, with results similar to those obtained for Case 8. The data are not included in Table 2 because there had been continuous occult blood

in the stools and it was felt that this rendered the reticulocyte data doubtful. This observation serves as a control to the others, and confirms our impression that *mucin, when mixed with iron in certain doses, will interfere with the absorption of the iron in the gastro-intestinal tract.*

Cases 9 and 10 serve as an additional control to the preceding observations. In these cases, iron was mixed with a large amount of cream and administered 3 times daily. The cream had no inhibiting effect on the utilization of iron as shown by the absence of second reticulocyte responses and of increased hemoglobin formation. The figures for the hemoglobin and reticulocytes are those which would be anticipated if iron had been daily without cream.

Comment. It cannot be proven definitely from these observations that an excess secretion of mucin in the gastro-intestinal tract will materially influence the absorption of iron. In the presence of achlorhydria, however, increased amounts of mucus in the gastric contents may play some rôle in the malabsorption of iron of the food, especially when the food is deficient in available iron. There is evidence that in cases of "idiopathic" hypochromic anemia with achlorhydria the response of the blood to iron is less marked than in cases of hypochromic anemia with normal acidity of the gastric contents. Excess mucus, which may accompany low acidity, is possibly a factor in this phenomenon.

The observation that cream apparently will not inhibit the utilization of small amounts of iron given by mouth, could possibly be duplicated by the study of other food substances: carbohydrates, protein substances, bulky foods. It is common knowledge, however, that in the routine treatment of patients, iron as well as many other sorts of medication may be tolerated better if administered after meals than before meals. Food may therefore delay or prolong the absorption of certain substances if not actually reduce the amount which can be absorbed.

The observations serve to demonstrate one of the many factors which can condition the absorption of iron and presumably other necessary food substances from the gastro-intestinal tract. The problem of absorption is extremely complicated, and the list of possible factors influencing absorption in the intestine is constantly growing.[3]

Conclusion. When iron in small doses is administered with relatively large amounts of mucin to cases of chronic hypochromic anemia (iron deficiency), its absorption from the intestine is inhibited.

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CLINICAL OBSERVATIONS ON THE WHIPPLE LIVER FRACTION (SECONDARY ANEMIA FRACTION).

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In 1930, Whipple, Robscheit-Robbins and Walden[21] reported a liver fraction which was potent in the treatment of experimental post-hemorrhagic anemia. Their experiments were performed upon carefully standardized dogs in which a chronic anemia had been produced by repeated bleeding. During the past 3 years the effect of this fraction of liver has been studied at the hospital of this institute in 11 patients with chronic hypochromic microcytic anemia.

The fraction is the dry, insoluble material obtained from the precipitation of an acidified aqueous extract of whole liver with 70% alcohol. It represents 3% by weight of the whole liver or 10% by weight of the dried liver. The fraction was found by Whipple and associates to possess 65 to 75% of the potency of whole liver as measured by new hemoglobin production in the standard anemic dogs. They demonstrated by control experiments with iron feeding, that the iron content of the liver fraction was not wholly responsible for the hemoglobin regeneration in these dogs. This fraction of liver has been shown to be practically inert in the treatment of patients with pernicious anemia,[3] and conversely the fraction of liver soluble in 70% alcohol (Fraction G of Cohn), containing the hematopoietic principle effective in pernicious anemia, possesses only 10 to 20% of the potency of whole liver in the treatment of Whipple's anemic dogs[17] and exerts little or no therapeutic action in the secondary anemias of man.[10,14]

Although the oral administration of whole liver and crude extracts of whole liver has proved useful in the treatment of human secondary anemias,[5,8,9,11,14,18,19] there have been few studies of the clinical effect of Whipple's fraction of liver (Eli Lilly and Company's, Liver Extract No. 55 with Iron); in this commercial preparation 0.5 gm. of iron and ammonium citrate (83 mg. iron as metal) has been added to each 3 gm. of the dry powder (the amount of extract derived from 100 gm. of whole liver). In 1932, Cheney and Niemand[2] published a series of 50 cases of secondary anemia treated with the commercial preparation. Excellent results were obtained in 13 cases of post-hemorrhagic anemia with rises in reticulocyte, hemoglobin and erythrocyte levels, whereas other forms of secondary anemia were not strikingly benefited. Since the patients of this series received 230 to 700 mg. of iron as metal daily mixed in with the liver extract, it is impossible to evaluate the efficacy of the liver extract apart from its iron content in these cases, even though the authors re-

ported that 6 of their post-hemorrhagic cases had failed to show material improvement on previous iron therapy. More recently Cheyney[1] has found the same commercial preparation of value in the treatment of certain cases of anemia secondary to gastric cancer.

In the course of their study of hookworm in Puerto Rico, Rhoads, Castle, Payne and Lawson[16] treated 7 patients with the No. 55 fraction without iron. On a daily dosage of 12 gm. of the extract 4 of the patients showed reticulocyte rises of from 3.6 to 13.4% followed by moderate improvement in hemoglobin and erythrocyte levels. The 12-gm. daily dose of the liver extract was found on analysis to contain 34 mg. of iron as metal which might be sufficient to exert some hematopoietic effect and thereby account at least in part for the apparent activity of the material. In all 7 cases secondary reticulocyte responses and more rapid improvement in hemoglobin and levels of erythrocytes followed the addition of 2 gm. of ferric ammonium citrate to the daily dose of liver extract.

The lack of conclusive proof that the clinical effect of the Whipple liver fraction is not entire due to the iron content stimulated the present clinical study. Heath[7] has stressed the individual variability in the iron requirement of patients with hypochromic microcytic anemia, pointing to one case which gave a maximal response to 85 mg. of iron a day by oral administration in contrast to 3 cases which showed no response whatsoever to a dose of 50 to 85 mg. per day given by the same route. Reimann and Fritsch[15] have reported good results with as little as 22 to 100 mg. of iron a day administered by mouth. It was in order to control the iron effect that the following method of procedure was adopted in the investigation of the clinical activity of the Whipple liver fraction.

Methods. Liver Extract No. 55, which was supplied without added iron by Eli Lilly and Company, was analyzed for its iron content by the method of Wong.[22] Several determinations revealed an iron content of 2.5 to 2.75 mg. of iron per gm. of dry powder, or 62 to 69 mg. of iron per 25-gm. dose of the extract.*

The principle of the double reticulocyte response was then employed in the study of the patients. This principle was introduced by Minot and his associates[11] to determine the optimal dosage of liver in pernicious anemia. Later Dameshek and Castle[4] used the method for assaying the relative potency of commercial liver extracts in the treatment of pernicious anemia, while Heath[7] has devised a similar comparative test for evaluating the efficacy of various iron preparations in the treatment of hypochromic anemia. As stated by Dameshek and Castle, the principle of the double reticulocyte response is briefly this: When a uniform daily suboptimal dose of a known potent material is given, the reticulocyte response is concluded or on its downward course in from 10 to 12 days. This allows for the observation of a possible second response of reticulocytes when another more potent material or a larger dose of the same material is given over a period immediately following the first period. Daily doses must be used since the stimulus to the bone marrow must be continuously applied during the estimation of the comparative potency of each product.

* We are indebted to Dr. Walther Goebel for the information that the iron in the liver fraction is chiefly in the ferrous state, although a small portion is ferric iron.

Applying this principle of the double reticulocyte response, we have treated patients with severe hypochromic microcytic anemia continuously over 3 consecutive periods of at least 10 days each. In the first period, each patient received about 70 mg. of iron a day as 1.7 cc. of a 25% solution of ferric ammonium citrate, in the second period 25 gm. of Liver Extract No. 55 (the amount derived from 833 gm. of whole liver) daily, and in the third period a maximal dose of inorganic iron preparation. Reticulocytes were followed throughout the first two periods in all cases, and through the third period in the majority of the cases. We have felt that after giving 70 mg. of iron a day throughout the first period we were justified in interpreting any further reticulocyte response occurring in the second period as due to some factor or factors in the liver fraction other than the contained iron. In other words, we have looked upon the first period as controlling the iron content of the liver fraction.

TABLE 1.—RETICULOCYTE PEAK OBTAINED WITH SMALL DOSES OF IRON, LIVER EXTRACT AND LARGE DOSES OF IRON.

Patient.	Sex.	Age.	Diagnosis.	Blood picture at start of test period.					Reticulocyte peak (%).		
				R.B.C. (in millions).	Hemoglobin (%).	M.C.V. (μ^3).	C.I.	Reticulocytes (%).	I. 70 mg. iron q.d.	II. L.E. No. 55 & iron, 25 gm. q.d.	III. Large dose of iron q.d.
1. F. P.	M	41	Ulc. colitis	4.05	37	58	0.46	0.8	1.6	4.0	11.2
2. H. B.	M	21	Ulc. colitis	3.18	46	73	0.72	1.4	6.0	12.5	
3. A. Z.	F	27	Ulc. colitis	2.61	30	69	0.58	2.0	7.0	6.8	12.1
4. E. T.	M	20	Banti's disease Hematemesis	4.60	56	61	0.61	1.8	4.2	5.6	
5. O. L.	F	45	Ca. of small intestine	2.43	41	78	0.84	0.6	4.0	9.8	9.0
6. E. W.	F	58	Ca. of cecum Achlorhydria	4.14	45	59	0.54	1.8	4.5	3.9	5.7
7. M. M.	F	29	Myomata uteri	3.82	51	68	0.67	0.4	2.4	15.8	8.0
8. M. B.	F	47	Gastro-enterostomy Hypochlorhydria	3.40	40	65	0.58	0.4	1.2	3.0	
9. C. L.	F	55	Hypochlorhydria	2.74	34	62	0.62	1.8	4.8	5.2	
10. H. P.	F	70	Nutritional anemia	3.31	43	70	0.65	1.2	5.4	5.2	3.0
11. L. B.	M	64	? Ca. of prostate; metastasis to bone	3.00	39	68	0.65	0.2	3.0	4.2	4.4
Average				3.39	42	66	0.63	1.2	4.0	6.9	7.6

Case Material. Eleven cases of severe hypochromic anemia (Table 1) were studied in the manner just described: 7 patients were females; 4 males. The ages varied from 20 to 70 years. Chronic blood loss was regarded as the chief etiologic factor in the first 7 patients, 3 of whom were suffering from chronic ulcerative colitis. Gross blood loss of sufficient magnitude to stimulate a reticulocyte response was not observed in any of these 7 patients during the period of observation. Cases 8 and 9 showed gastric hypochlorhydria, associated with a gastro-enterostomy in Case 8. Case 10 presented the typical picture of nutritonal anemia resulting from an inadequate iron intake over a period of years, while Case 11 was a man with carcinoma (probably of the prostate) with multiple metastases to bone.

The original erythrocyte levels of the 11 patients varied from 2,430,000 to 4,600,000 red blood cells per c.mm., while the hemoglobin levels ranged from 30 to 56%. In 9 cases the hemoglobin level was below 50%. The mean corpuscular volume and color index were well below normal in all

11 cases, averaging 66 μ^3 and 0.63 respectively. The reticulocyte levels at the start of the observation period varied from 0.2 to 2%. Thus these 11 patients all satisfied the usual criteria of hypochromic microcytic anemia with reticulocytes stabilized at a low level when the therapeutic tests were started.

Results. Eight of the 11 patients showed at least a slight reticulocyte response to the oral administration of 70 mg. of iron a day during the control period. These responses varied from 1.2 to 7% and occurred between the fourth and tenth day after the initial dose of iron. Following the peak, the reticulocyte curve either declined or flattened out until 25 gm. of Liver Extract No. 55 daily were substituted for the inorganic iron preparation in the second observation period. Then each of the 11 cases gave a secondary reticulocyte response to this liver fraction, the peak of the rise ranging from 3 to 15.8%. A moderate rise in levels of erythrocytes and hemoglobin occurred during these first two periods of therapy; however, the rate of improvement in blood levels was much more rapid in the third period when the patients were receiving large doses of inorganic iron. Of the 7 cases where reticulocytes were followed throughout the third period 5 developed tertiary reticulocyte rises which equalled or excelled the primary and secondary responses. Examples of the blood changes taking place during the

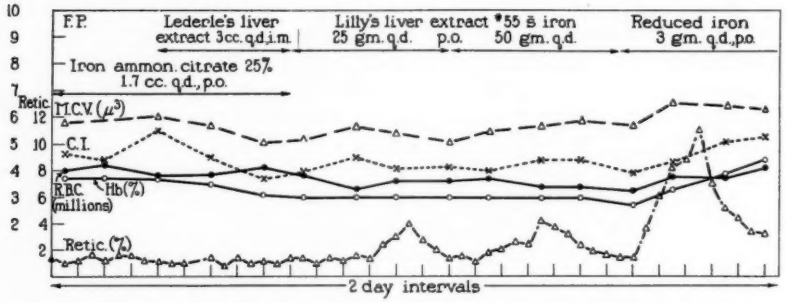

FIG. 1.—Case 1. F. P., male, aged 41. Chronic ulcerative colitis. No reticulocyte response to 70 mg. of iron a day or to antipernicious anemia liver fraction. Slight reticulocyte response to "secondary anemia" liver fraction and excellent response to large doses of reduced iron.

three consecutive test periods are presented in Figures 1 to 6, while Figure 7 and Table 1 summarize the data for the entire series of 11 cases.

Discussion. It is quite apparent from examination of Table 1 and the text figures that all 11 cases of hypochromic microcytic anemia showed at least slight secondary reticulocyte responses to the No. 55 liver extract, which averaged 2.9% higher than the original response to the 70-mg. dose of iron. These figures suggest that the secondary anemia liver extract contains clinically active hematopoietic material

apart from its iron content. The nature of this material is not known. Patek and Minot[13] have described reticulocyte responses to the oral adminstration of bile pigment following a period of iron medication in cases of hypochromic microcytic anemia, and Patek[12] has reported similar reactions to the administraton of chlorophyll

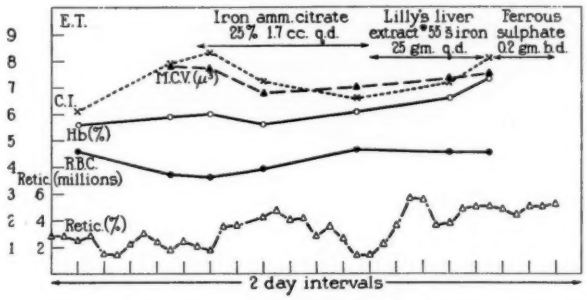

FIG. 2.—Case 4. E. T., male, aged 20. Banti's disease. Large hemorrhage 3 weeks before admission. Reticulocyte rise to 4.2% during control period; secondary rise to 5.6% during liver extract period.

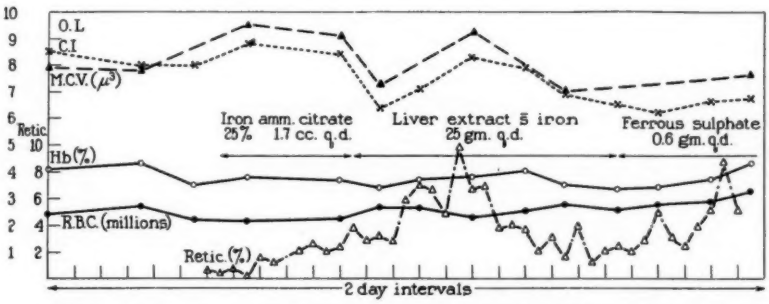

FIG. 3.—Case 5. O. L., female, aged 45. Carcinoma of small intestine. Reticulocyte rise to 4% during iron control period; further rise to 9.8% during liver extract period; tertiary response of 9% to 0.6 gm. of ferrous sulphate daily.

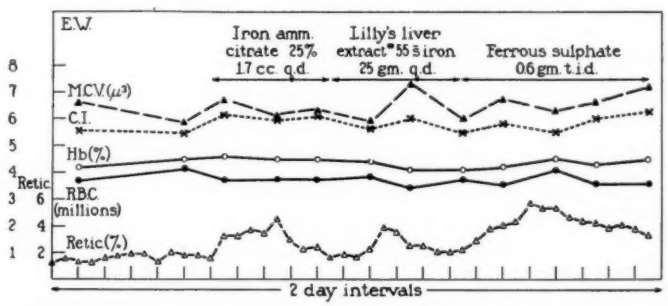

FIG. 4.—Case 6. E. W., female, aged 58. Carcinoma of cecum, histamine achlorhydria. Slight reticulocyte rises in each of the three consecutive observation periods without appreciable improvement in erythrocyte or hemoglobin levels.

products following a suboptimal dosage of iron. These observations have been interpreted as evidence that the human body may utilize the pyrrols present in bile pigment and in chlorophyll for the formation of new hemoglobin. As early as 1925 Whipple and Robscheit-Robbins[20] concluded that parent pigment substances are stored in the normal liver. It may be that the No. 55 liver fraction supplies

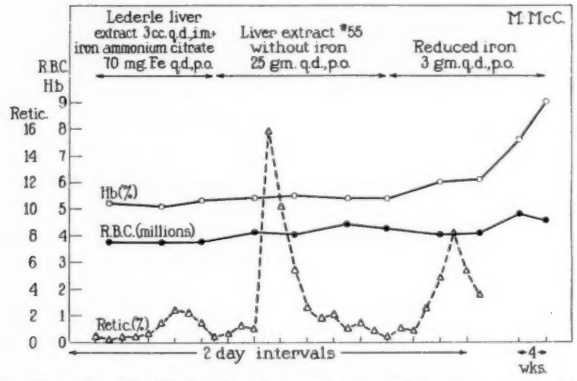

FIG. 5.—Case 7. M. McC., female, aged 29. Myomata uteri with previous hemorrhage. No blood loss during test period. Slight reticulocyte response to 70 mg. of iron a day; sharp secondary reticulocyte rise to 15.8% on the liver extract; moderate tertiary response to reduced iron in large dosage followed by rapid rise in hemoglobin.

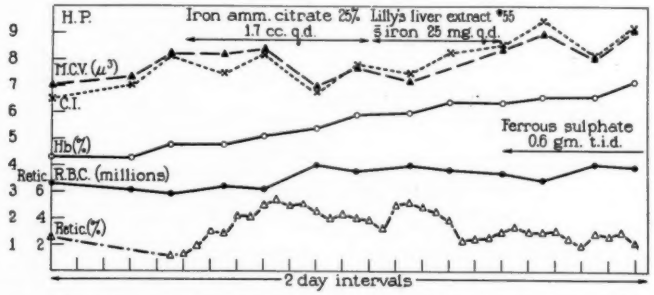

FIG. 6.—Case 10. H. P., female, aged 70. Nutritional anemia. Reticulocyte response of 5.4% to 70 mg. of iron and secondary response of 5.2% to the liver fraction. Steady rise in hemoglobin level.

such parent pigment substances for new hemoglobin formation. On the other hand, it is conceivable that this liver fraction may improve the absorption or utilization of iron.

In view of the fact that the iron contained in the liver fraction is chiefly ferrous iron whereas ferric iron was administered in the control period, it is possible that the secondary reticulocyte rise may be attributed to the recognized superiority of ferrous over ferric iron in the treatment of hyperchromic anemia. How much

of the iron contained in the liver fraction is available and absorbed is not known.

Hart *et al.*[6] have shown that the effect of whole liver or liver extracts on nutritional anemia of the rat produced by a diet of milk is directly proportional to the available content to iron and copper. The iron-copper ratio of the liver fraction used in our experiments is not known. It is possible that the iron-copper ratio was brought more nearly to normal by the administration of the liver fraction; this may be the explanation of the reticulocyte rise. Our present studies furnish no data to substantiate or disprove any one of these hypotheses as to the mechanism of action of the liver fraction.

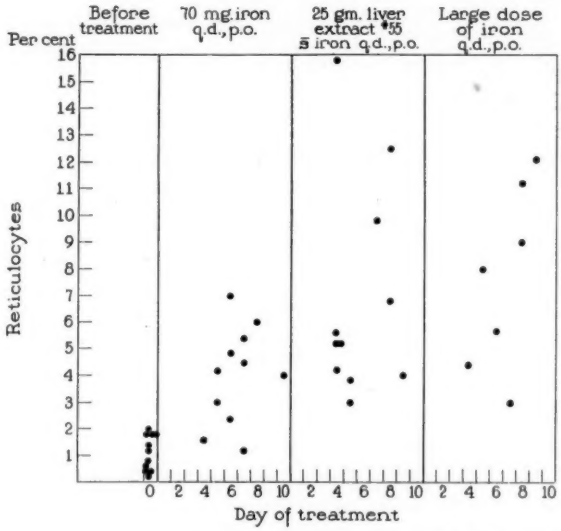
Before treatment
70 mg. iron q.d., p.o.
25 gm. liver extract +55 mg. iron q.d., p.o.
Large dose of iron q.d., p.o.
Per cent
Reticulocytes
Day of treatment

FIG. 7.—Composite chart of 11 cases. Reticulocyte peaks plotted against days on which these peaks occurred during the three consecutive observation periods.

The tertiary reticulocyte responses to maximal doses of inorganic iron and the more rapid improvement in blood levels on this form of treatment may be regarded as further evidence that iron in adequate amounts is the most effective therapeutic agent in the hypochromic microcytic anemias of man.

Summary and Conclusions. The secondary anemia liver fraction of Whipple and his coworkers has been administered orally to 11 patients with chronic hypochromic microcytic anemia. A rise in circulating reticulocytes occurred in every case.

Through the application of the principle of the double reticulocyte response to control the iron content of the liver fraction, it appears likely that the liver fraction contains reticulocytogenic material apart from its iron content.

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PULMONARY INVOLVEMENT IN LYMPHOSARCOMA AND LYMPHATIC LEUKEMIA.*

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We published a paper, in 1935,[3] concerning Hodgkin's disease of the lung. At that time studies were planned to include pulmonary involvement in two other members of the so-called lymphoma or lymphomatoid[5] group of diseases, lymphosarcoma and lymphatic leukemia. This report concerns our data on the latter two diseases.

The material is from patients admitted to the University of California Hospital from 1920 to 1937, inclusive. Our studies attempt to correlate the clinical, pathologic, and roentgenologic findings in those cases showing pulmonary involvement. Under pulmonary involvement, we include bronchial and hilar lymph nodes, pleura, and parenchyma of the lungs. In no case in either series is the involvement limited to bronchial and hilar lymph nodes with the possible exception of Case 10, Table 2. An autopsy could not be obtained for verification of parenchymal involvement of the lung in this case.

Table 1.

Authors.	Cases lympho-sarcoma.	Pulmonary involve-ment.	Per cent.	Cases lymphatic leukemia.	Pulmonary involve-ment.	Per cent.
Kirklin and Hefke . .	84	17	20	48	10	21
Falconer and Leonard .	25	9	36	30	9	30

* Read before the General Medicine Section of the California Medical Association at the 66th Annual Session, Del Monte, May 2 to 6, 1937.

Table 2.—Data of Cases of Lymphatic Leukemia With Pulmonary Involvement.

Initials.	From onset of disease to	Duration of life after

TABLE 2.—DATA OF CASES OF LYMPHATIC LEUKEMIA WITH PULMONARY INVOLVEMENT.

Case No.	Initials. Sex. Age (yrs.).	From onset of disease to appearance of pulmonary symptoms (mos.).	Duration of life after pulmonary involvement (mos.).	Roentgen findings.	Roentgen therapy.	Biopsy, autopsy or other result.	Comments on pathologic findings.
1	M. J. W. M 26	Primary pulmonary onset	2	Fluid left chest	None	No biopsy. Blood studies indicated lymph. leuk.; also autopsy	Tumor masses reaching thymus and involv. hilar nodes, both lungs, and upper lobe left lung.
2	E. H. M 19	18	1	Bilateral pleural effusion	None	Sternal bone marrow. Differential blood counts. Autopsy revealed aleuk. and lymph. leuk.	Bilateral pleural effusion. Interstitial pulmonary infiltration with lymphoblasts and lymphocytes
3	W. M. M 63	9	7	Dense hilar shadows. Prominent beaded pulmonary markings	Deep	No biopsy. WBC 163,000; 8% lymphocytes. No autopsy	Miliary disseminations through both lungs
4	E. R. M 56	2½	3½	No record	None	No biopsy. WBC 488,000; 97% lymphocytes. Lymph. leuk., generalized lymph. hyperplasia	Chylous hydrothorax on left
5	F. C. F. M 79	12	1	Pleural effusion on left	None	WBC 295,000; 50% lymphocytes; 45% lymphoblasts. Autopsy: lymph. leuk., leuk. infiltr. of lungs	Lungs show perivascular leukemic infiltration, also chr. fibrous tb.
6	W. C. M 69	36	1	Pleural effusion on left	None	None. WBC 840,000; 98% lymphocytes. Pleural effusion on left; enlarged hilar lymph nodes	Small amount fluid on right. Histologic picture—diffuse cellular pulm. infiltration
7	T. H. M 53	11	20	Hilar lymph node enlargement and tumor masses in mediastinum	Deep over chest and superficial nodes; good results	Cervical node Jan., 1931. Axillary node, April, 1931. Died of lobar pneu. elsewhere. Partial autopsy	Autopsy findings not conclusive. Clin. and lab. findings favor diagnosis aleuk. lymph. leuk.
8	J. C. M 70	24	24	Jan., 1933: chest neg. Aug., 1934: Bronch. infiltration, right, middle of lower lobe, also nodular areas	Deep to abdomen and chest	Cervical node, 5-18-34. Diag. lymph. leuk. WBC 40,000 to 102,500 majority of cells lymphocytes. Bronchial nodes, enlarged right lower, show extensive involvement. Bilat. pleural effusion	Extensive involvement parenchyma right lung and pleura
9	H. G. M 64	132	4	Hilar lymph node involvement	Several courses of deep	Sternal biopsy. WBC 20,000 to 283,000, greater portion are lymphocytes. Involv. hilar nodes with infiltration of peribronchial tissue	Long duration of disease with pulm. involv. late in course of disease. Diffuse pulm. infiltration microscopically
10	G. H. M 53	36	1	Large mass in anterior portion of right hilus compressing superior vena cava	Chest and nodes. WBC fell from 900,000 to 300,000	Axillary lymph node showed lymph. leuk. Blood films indicated lymph. leuk. No autopsy. Sternal marrow biopsy: 90% large and small lymphocytes	Marked venous engorgement, vessels of neck, upper chest, both anterior shoulder regions. Dyspnea marked
11	W. R. M 23	Primary	2½	Widened mediastinal shadow, right pleural effusion	Three courses to chest	Biopsy of imbedded pleural fluid sediment diagnosed lymphoblastoma	Probable prim. pleu. involvement with sec. involv. of mediastinal nodes. Nodes soft, almost caseous, first thought to be tb.

TABLE 3.—DATA OF CASES OF LYMPHOSARCOMA WITH PULMONARY INVOLVEMENT.

Case No.	Initials. Sex. Age (yrs.).	From onset of disease to appearance of pulmonary symptoms (mos.).	Duration of life after pulmonary involvement (mos.).	Roentgen findings.	Roentgen therapy.	Biopsy, autopsy or other result.	Comments on pathologic findings.
12	M. J. F 16	Primary pulmonary onset	4	Pleural effusion on left	Not well tolerated	Cervical lymph node: lymphosarcoma. At autopsy left pleura greatly thickened and infiltr. with tumor cells	Diffuse tumor infilt. left lung. Diaphragm adherent to left lung. No mediastinal lymph node involv.
13	M. S. F 42	36	3	Pleural effusion on right	Courses from Aug., 1922, to May, 1923	Biopsy from tissue removed at abdominal operation: lymphosarcoma	Microscopic study: diffuse infilt. into lung tissue of small, discrete, nodular type
14	M. E. F 56	4	1	"Chronic fibrous tb., right lung"	Radium and Roent. used	Skin-biopsy: diag. mycosis fungoides. Autopsy: localized nodules both lungs. Tumor mass, apex left lung	Microscopic examination: localized areas of infiltration replacing normal lung structure
15	J. T. M 53	5	Terminal	Chest film pronounced negative Nov. 16, 1931	17 treatments. Response poor	Cervical lymph node diagnosis: reticulum cell sarcoma. Autopsy: coëxisting healed pulmonary tb. Serosanguinous fluid both pleural sacs.	Microscopic findings: circumscribed collections of tumor cells scattered through lower lobes, both lungs. Mediastinal nodes involved
16	J. J. M 39	13	3	Sept. 9, 1933: diffuse mottling through both lungs suggesting tb.	Irrad. over 2 mos. period, 1933. Again few treatments in hosp. before he became too ill	Cervical node: lymphosarcoma. At autopsy: bilat. pleu. effusion. Both lungs diffuse infiltration	Microscopic findings: peribronchial and perivascular infiltration in lungs. Pleura thickened and studded with small tumor nodules
17	G. H.* M 45	24	5	Large mass left lung, occupying upper half mediastinum on left	Large mass receded under treatment	No biopsy. Autopsy: small bilat. mediastinal tumor; upper lobe left lung, extensive scar tissue replacement	Left pleural cavity obliterated by extensive fibrous tissue. Microscopic study: fibrous tissue infiltrated by tumor cells

18	M. A. F 34	24	4	Right pleural effusion	Several courses with good response at first	Cervical node tumor and rib biopsies: diag. Hodgkin's dis. Autopsy: extensive involv. throughout entire body. Fluid in both pleural sacs	Hilar and mediastinal lymph nodes involved. Pleural adhesions and nodular infiltration
19	D. R. M 60	1	1	Diffuse shadow base of left lung suggesting malignancy	Unsatisfactory response	No biopsy. Autopsy: bilat. pleural effusion, mediastinal lymph nodes enlarged	Left lung adherent to diaphragm. Diffuse pulmonary infiltration
20	L. J. J. M 68	6	8 (still under observ.)	Lymphatic infiltration stomach, chest film 12-20-36; also enlarged hilar nodes, right pleural effusion	Two courses, 1936; one, 17; one, 12 treatments	Biopsy, cervical lymph node: lymphosarcoma	Disease spread cervical region to stomach and abdomen, then chest, neck, parotid region and eyelids with Mikulicz's syndrome. Response to Roentgen therapy good
21	D. K. F 53	36	12	Right hilar nodes enlarged, lower portion right lung discrete nodules, extensive infiltration left lung with nodules	Only fair response	Biopsy cervical lymph node: lymphosarcoma. Autopsy: in right lung, large, solid mass involving upper and middle lobe. Lung tissue destroyed and replaced by fibrous tissue	Large white mass in right side of mediastinum. Multiple small nodules lower lobe. Left lung, mass upper lobe, nodules lower lobe
22	E. C. M 30	Primary	7 (still under observ.)	Pleural effusion left. Huge masses of glands in posterior mediastinum	Satisfactory response	Biopsy left axillary lymph node: lymphosarcoma	Bilateral pleural effusion. Venous obstruction upper chest and neck. Example of paratracheal node involvement
23	E. J. F 27	24	48 (still living)	Mass involving upper lobe right lung, compressing trachea	Fair response	Two biopsies cervical lymph nodes: lymphosarcoma. Upper mediastinum filled by mass, marked obstruction to breathing	Right chest filled with fluid reported to have become purulent. Patient under treatment in Germany

* We are indebted to Drs. George Weaver of Stockton, Calif., and Sydney Shipman of San Francisco, Calif., for permission to use the data concerning Case No. 17 (G. H.).

Incidence.—Search of the literature for statistics of incidence showed surprisingly few reports. Those found are concerned for the most part with the roentgenologic aspects. Cutler[2] studied 30 patients with lymphosarcoma, 13 of whom came to autopsy. His statistics of pulmonary involvement are listed under separate headings—mediastinum, pleura, and parenchyma of the lung. Nine showed involvement of mediastinum; 7, pleura; and 3, parenchyma of the lung. Two recent papers dealing with the roentgenologic aspects of Hodgkin's disease and lymphosarcoma, are those of Williams,[9] and Pierce, Jacox, and Hildreth.[7] Not all of the cases in the respective series of these authors are proven by biopsy. Kirklin and Hefke[4] have reported an interesting study, from the roentgenologic aspects, of Hodgkin's disease, lymphosarcoma and lymphatic leukemia. These studies were made upon patients at the Mayo Clinic. All of their cases are proven by biopsy, with the possible exception of the lymphatic leukemia patients. Statistics of the Kirklin and Hefke series appear below and show a lower incidence of pulmonary involvement than our statistics. This applies to both lymphatic leukemia and lymphosarcoma, and is readily explained by the fact that Kirklin and Hefke's patients were under observation for relatively short periods and autopsy findings were not available in the majority of cases.

The true incidence of pulmonary involvement must rest largely on statistics based upon postmortem examination from the very nature of the two diseases. Beginning as local processes, both diseases spread throughout the body, until at autopsy there is wide dissemination. Exceptions to this usual course of development are occasional cases, in both disease groups, where the process remains relatively localized.

In our patients with lymphosarcoma where the process has been localized to the more superficial groups of lymph nodes, Roentgen films of the chest have been negative. Our observations in the lymphatic leukemia group are too few to permit of any conclusion.

Pathologic Anatomy. In contradistinction to the findings in Hodgkin's disease (Moolten[6]), primary involvement of pulmonary parenchyma in lymphosarcoma and lymphatic leukemia must be rare. We found no reference to this particular phase of study in the literature. No cases were found in our present study where clinical and pathologic evidence suggested inception of the disease in the parenchyma of the lung. Bernard[1] considers primary pleural involvement in Hodgkin's disease more common than primary manifestation in the parenchyma of the lung. This accords with our observations in the two diseases of the present study. The term "primary pleural involvement," is used here in a rather loose manner, as it is exceedingly difficult, if not impossible, in a given case to discover where lymphatic leukemia or lymphosarcoma has its earliest inception, just as it is often quite impossible to say where the initial

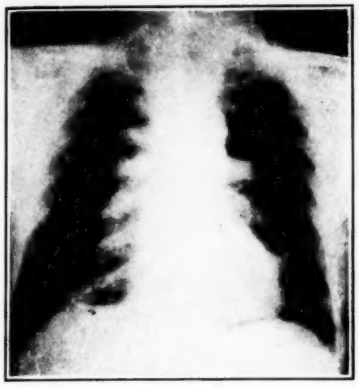

FIG. 1

FIG. 2

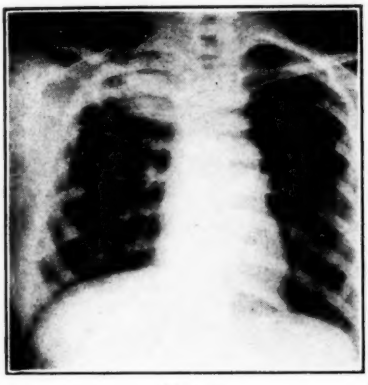

FIG. 3

FIG. 4

FIGS. 1 and 2, lymphatic leukemia; contrast with Figs. 3 and 4, lymphosarcoma. Note left paratracheal node involvement in both patients. Fig. 1 is almost identical with Fig. 3 of Williams' article, Fig. 4 illustrating "typical" paratracheal node involvement characteristic of Hodgkin's disease. Lateral views illustrate why these patients had marked venous compression. (Patients 10 and 23, Tables 2 and 3.) Barium in esophagus.

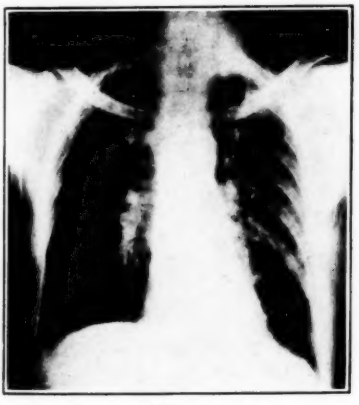

FIG. 5

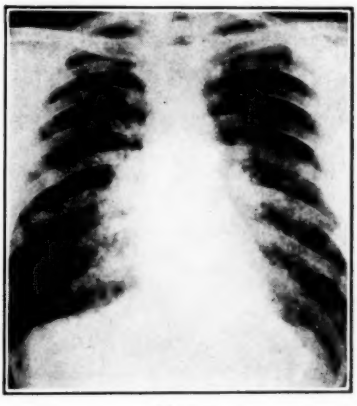

FIG. 6

FIG. 5, lymphatic leukemia; Fig. 6, lymphosarcoma. Illustrative of miliary type of distribution of pulmonary lesions. Responded to Roentgen therapy by rapid clearing of lungs, but progress of disease in both instances unchecked. (Patients 3 and 16, Tables 2 and 3.)

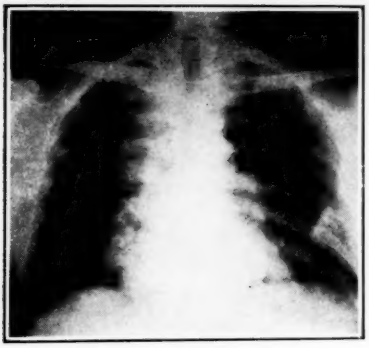

FIG. 7

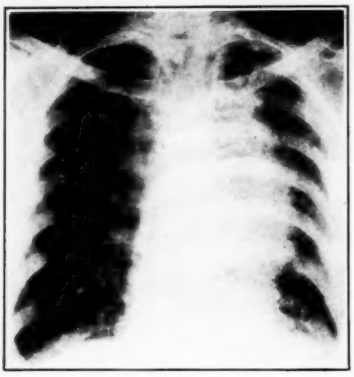

FIG. 8

FIG. 7, lymphatic leukemia; Fig. 8, lymphosarcoma. Note massive infiltration of lung parenchyma. Fig. 7 shows "feathering out" appearance at left margin of tumor, supposed to be characteristic of Hodgkin's disease. Pulmonary lesions receded under Roentgen therapy. Tumor tissue at autopsy found replaced by extensive fibrosis. Fig. 8, diagnosed originally as bronchogenic carcinoma from Roentgen films and bronchoscopy. (Patients 8 and 17, Tables 2 and 3.)

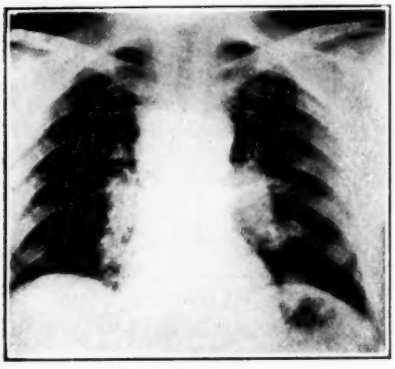

FIG. 9

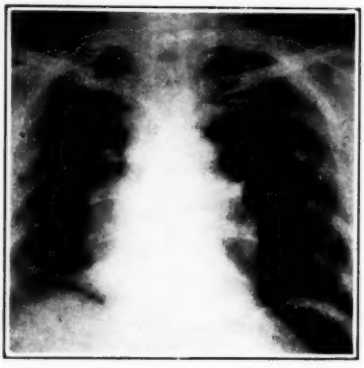

FIG. 10

FIG. 9, lymphatic leukemia; Fig. 10, lymphosarcoma. Hilus node involvement very similar in each case. Fig. 10 shows a small pleural effusion, right, which cleared rapidly under Roentgen therapy. (Patients 7 and 20, Tables 2 and 3.)

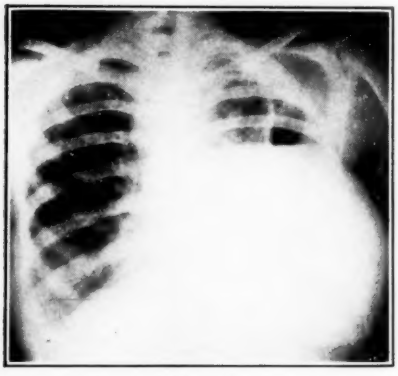

FIG. 11

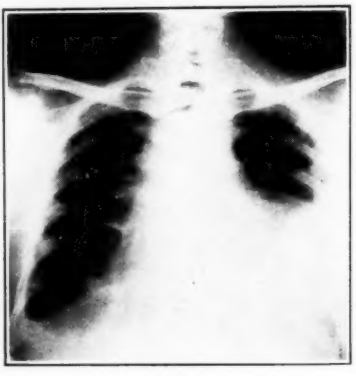

FIG. 12

FIGS. 11 and 12.—Lymphosarcoma. Fig. 11 probably primary pleural involvement. Fig. 12 showed primary hilar node involvement; pleural involvement appeared early. Fig. 11, note thickened pleura on left after introduction of air in pleural sac. (Patients 11 and 22, Table 3.)

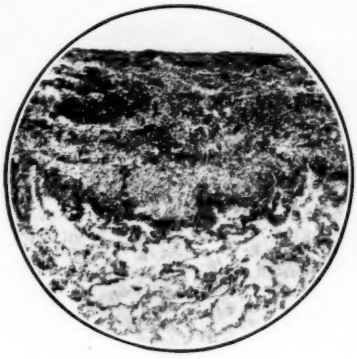

FIG. 13

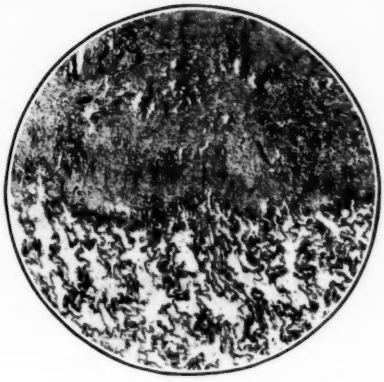

FIG. 14

FIGS. 13 and 14.—Lymphatic leukemia, and lymphosarcoma. Sections through pleura, showing normal lung tissue beneath. Note similar, remarkable, fibrous thickening of pleura in both cases. Extensive tumor cell infiltration. (Cases 11 and 12, Tables 2 and 3.) Probably primary pleural involvement.

FIG. 15

FIG. 16

FIGS. 15 and 16.—Lymphatic leukemia, and lymphosarcoma. Note similarity in type of pulmonary invasion. In both figures tumor nodule shows cross-section of an artery. Note perivascular infiltration. (Cases 6 and 15, Tables 2 and 3.)

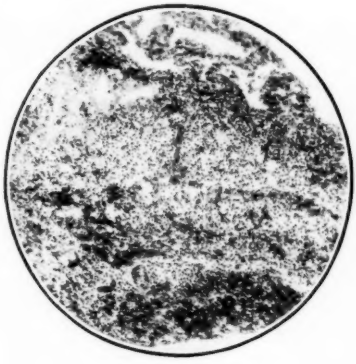

FIG. 17

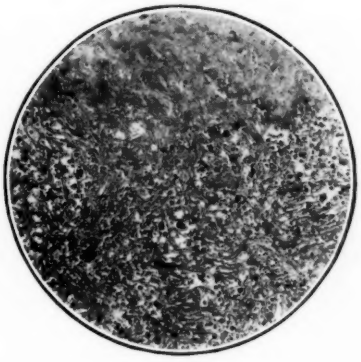

FIG. 18

FIG. 17.—Details of leukemic infiltration of pleura. Note uniformity of cells. (Case 11, Table 3.)

FIG. 18.—Extensive invasion of lung tissue by lymphosarcoma. Note pleomorphism of cells, also replacement of tumor by connective tissue. (Case 21, Table 3.)

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lesion of pulmonary tuberculosis is located. In this connection, however, it is interesting that in Cases 11 (Table 2) and 12 (Table 3) (lymphatic leukemia and lymphosarcoma) the involvement was apparently first pleural, then in the hilar lymph nodes, a situation comparable to what supposedly occurs in certain cases of pulmonary tuberculosis. Case 22 (Table 3) showed the first clinical signs of his disease (lymphosarcoma) in the mediastinum and pleura. As this patient is still under observation, nothing definite can be said concerning the initial lesion.

In grouping our patients in both series according to type of pathologic involvement, Versé's[8] classification is used. Table 4 shows the percentage for the different types, in both series of diseases.

TABLE 4 (VERSÉ).—SEPARATION INTO 5 TYPES ACCORDING TO LOCATION OF LUNG INVOLVEMENT.

Lymphatic leukemia.						Lymphosarcoma.					
I.	II.	III.	IV.	V.	Total.	I.	II.	III.	IV.	V.	Total.
5	1	2	2	2	12	3	3	1	0	4	11

This method of separating different types of pulmonary involvement was originally suggested for Hodgkin's disease by Versé, who described the types as follows:

"I. Hilar node involvement with direct invasion of lung tissue: (*a*) By direct extension from the lung hilus; (*b*) by breaking through the mediastinal pleura. II. Hilar node involvement with intra- and peribronchial spread into the lungs. III. More or less lobar infiltration with various grades of bronchomediastinal involvement. IV. Confluent lobular foci with associated involvement of lymph nodes. V. Miliary disseminations."

Pleural effusion was present in a high percentage of our series, in both diseases. The lymphatic leukemia group showed 64%, 7 of the 11 patients. In the lymphosarcoma group the figure was slightly higher, 67%, *i. e.*, 8 of the 12 patients in the series. Of the 7 pleural effusions in the first group, 2 were bilateral, 3 left sided, 2 right sided. The 8 cases with effusion in the lymphosarcoma group showed bilateral 5, right sided 2, left sided 1. In our series of cases of Hodgkin's disease,[3] 71% of the series (7 patients) showed pleural effusion, probably too high a percentage, owing to the small number in the series.

This high incidence of pleural effusion is striking, and stresses the importance of Roentgen films of the chest at regular intervals, particularly when signs and symptoms indicate progression of the disease. It is our impression from analysis of these series, that cases with massive pleural effusion respond unsatisfactorily to Roentgen therapy.

Patients 12 (Table 3) and 11 (Table 2) lymphosarcoma and lymphatic leukemia, are probably cases with primary pleural involvement. The earliest symptoms were referable to the chest.

Pleural effusion was found shortly after the onset of symptoms. In both instances the microscopic picture of the pleura, showing considerable fibrous tissue replacement, is indicative of early pleural involvement, particularly as the entire length of disease was only 10 and 16 weeks, respectively.

Roentgen Studies. In our studies on Hodgkin's disease,[3] we came to the conclusion that there was no characteristic Roengten picture of pulmonary Hodgkin's disease. The same conclusion is obvious from our studies on lymphosarcoma and lymphatic leukemia. A similar opinion is expressed by Kirklin and Hefke.[4] They state that roentgenologic data at the present time do not permit of an exact differential diagnosis. To quote from their article: "Any of the three diseases may exactly resemble either of the other two, and in view of their morbid anatomy, this might be expected."

It is not within the scope of this report to discuss results of Roentgen therapy. For those interested we might state our impression, that Roentgen therapy accomplished no more than symptomatic relief in our series of patients. The cases were, with few exceptions, the less favorable types, with steady downward progression, and when pulmonary involvement occurred, the disease was well implanted in other portions of the body.

Prognosis. In the lymphatic leukemia group, the duration of life was 2 months to 11 years, 8 patients living less than 4 years after onset of symptoms. In the lymphosarcoma series the figures for duration of life are 4 months to 4½ years, 8 living less than 4 years after onset of symptoms. Pulmonary involvement in the majority of patients in both series tended to occur rather late in the course of the disease or more precisely speaking, it tended to occur when the disease became widespread in the body. Exceptions to this finding occurred and have been discussed under pleural involvement. Once the parenchyma of the lung becomes involved or pleural effusion supervenes, the outlook, both as to tenure of life and induction of remissions by Roentgen therapy, is poor. This conclusion appears warranted from our studies, but it cannot in any sense be accepted as a rule to be followed. Patients with either disease under discussion vary greatly in their sensitivity to radiation, and for this reason it should always be used to the full limits of its possibilities.

Summary. The figures for pulmonary incidence were: lymphatic leukemia, 30%; lymphosarcoma, 36%. Our findings in Hodgkin's disease showed 31%. These figures enhance in interest if one is to consider Hodgkin's disease an infection, and the former two diseases neoplastic in nature. From the clinical, roentgenologic and pathologic aspects, our studies with reference to pulmonary involvement in the 3 groups of diseases showed a striking similarity. There were, of course, some minor differences, for example, the difference in the grouping of cases under the Versé classification. However, this grouping depends largely on individual interpretation of the

pathologic findings and will vary according to the extent and thoroughness of the microscopic study of the tissues at necropsy.

In our series of cases of Hodgkin's disease,[3] the time between onset of disease and appearance of pulmonary symptoms varied from onset with pulmonary symptoms to 8 years. In 2 patients pulmonary symptoms were primary. In the other 5 patients of the series, the time varied from 2 months to 8 years. Length of life after onset of pulmonary involvement varied from 22 months to 4 years and 3 months. These figures, when compared with the corresponding figures for lymphatic leukemia and lymphosarcoma, show that the patients of our Hodgkin's disease series apparently ran a more slowly progressive and chronic course than the cases comprising the two series of the present study. The importance of this study, along with our study of Hodgkin's disease, is to point out the similarity, both as to pulmonary incidence and types of involvement, in these three diseases, which are frequently included under a generic term: lymphoblastoma, lymphoma, or lymphomatoid[5] group of diseases.

We wish to thank Dr. C. L. Connor, Chief of the Department of Pathology, for his suggestions and advice. We are also indebted to Drs. Robert Stone and C. S. Capp of the Department of Roentgenology for the interpretation of the roentgenograms used in this study.

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ELECTROCARDIOGRAPHIC CHANGES AND PERIPHERAL NERVE PALSIES IN TOXIC DIPHTHERIA.

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It is our purpose to record the results of an investigation into the clinical, electrocardiographic and pathologic changes due to cardiac complications in toxic diphtheria. Peripheral nerve palsies

closely parallel these complications, and data relative to their occurrence are included in this study.

In 1900, Councilman, Mallory and Pearce[2] described the tissue changes in 220 patients dying of diphtheria. Extensive histologic studies on fresh and fixed sections of the myocardium were made on 60 of them. Of this latter group, 36 showed fatty degeneration of the muscle fibers. The more advanced changes consisted of progressive necrosis leading to complete destruction of the fibers. They found little relationship between the duration of the disease and the presence of fatty degeneration. This type of degeneration was generally the only finding of great severity in the patients who died early in the disease. They found, however, a definite relationship between the intense degenerative changes (necrosis) and the duration of the diphtheritic infection.

Warthin,[8] Marvin,[4] Nathanson,[5] Strecker,[7] Alstead[1] and others described the changes found in electrocardiograms on patients suffering from toxic diphtheria. These alterations consist of depression of the $S-T$ line, inversion of the T wave, intraventricular block and auriculoventricular dissociation.

Josephthal[3] produced electrocardiographic changes in the rabbit, analogous to the changes encountered in toxic diphtheria, by injecting sublethal doses of diphtheria toxin. The records on the rabbits showed the progressive development of negative T waves and intraventricular block.

Clinically, the mortality in diphtheria is credited for the most part to myocardial failure, and the average death certificate reads: contributory cause, toxic myocarditis.

Our studies attempt to correlate the cardiac changes as determined by clinical observation, serial electrocardiograms and autopsy material. These observations serve to demonstrate the pathogenesis of this complication by recording the time of onset and the progress of myocardial changes during the course of the primary diphtheritic infection.

The methods of investigation adopted comprised the taking of electrocardiograms on patients showing membranes and toxic manifestations of diphtheria. The graphs were taken soon after admission and repeated every second or third day during the course in the hospital. Tracings were taken daily or twice daily on patients showing marked changes in the contour of the electrocardiograms.

Early in the course of our studies, peripheral nerve palsies seemed to occur with unusual frequency in the patients who showed abnormalities in the electrocardiograms. Because of this fact, the date of their occurrence, in the course of the illness, was recorded to determine whether they were in any way related to the cardiac manifestations.

Although this investigation was carried on for approximately $2\frac{1}{2}$ years, and included some 140 cases of toxic diphtheria, only 28

showed electrocardiographic evidence of cardiac damage. This latter group comprise the basis of the present study together with some additional pathologic material taken from the autopsy records of the hospital.

The Electrocardiographic Changes. All patients admitted early in the disease yielded normal electrocardiograms except for the presence of tachycardias. The abnormal features, which developed during the subsequent course of the disease, were divisable into the following two groups: 1, Alterations in the *T* wave; 2, alterations in the conduction system, including auriculoventricular block and intraventricular block.

The T-wave Variations. The earliest change noted, on the electrocardiogram, was a slight decline in the *S–T* line and a decreased amplitude of the *T* wave. It soon became evident that the occurrence of this change portended the development of more radical alterations. The *T* wave generally fell progressively until it became isoelectric, 1 or 2 days later it would become diphasic and subsequently inverted. The *T* wave returned to normal in the reverse order of events. This phenomenon occurred in all 3 leads, but it was most pronounced in the second lead.

Of 23 patients showing these changes, the earliest occurred on the fifth day of illness and the latest on the thirty-ninth day, with

TABLE 1.—TOXIC DIPHTHERIA CASES SHOWING ELECTROCARDIOGRAPHIC CHANGES IN THE *T* WAVE.

Case No.	Age.	Days from onset of illness to antitoxin.	Amount of antitoxin in 1000 units.	Days from onset to *T*-wave change.	Duration of *T*-wave change.	Days from onset to peripheral nerve paralysis.	Comment.
5	28	4	50	15	36	30	Normal on discharge.
7	11	4	40	15	20	10	Normal on discharge and 1 yr. later.
12	2	8	50	8	1	...	Died; autopsy.
16	4	6	38	24	12	27	Also conduction change 9th day.
17	19	3	82	*	*	32	Died suddenly 61st day of illness.
19	33	3	40	13	7		
26	8	5	40	10	44	14	
27	7	2	40	27	6	8	
30	25	7	50	14	7		
34	18	(9) none	None	9	72	38	
37	36	7	80	21	38	45	
41	5	4	30	18	7	32	Also conduction change 8th day.
54	6	6	40	9	59	27	
69	57	6	25	14	26	35	Died; autopsy.
76	2	3	35	39	1		
77	4	(9) none	None	7	25	...	Ventricular escape.
78	53	5	35	8	1	16	
97	10	6	30	11	25	9	
103	24	4	45	5	13		
105	38	3	40	16	43	20	
124	26	6	50	19	19	26	
126	6	3	20	9	47	21	
127	4	4	30	10	38	11	
Average	18	5	42	15	24	23	

* Records inadequate.

an average onset on the fifteenth day. The change was noted to persist for various periods of time ranging from 1 to 72 days; the average duration was 24 days (Table 1 and Case Histories).

Shookhoff and Taran[6] noted similar *T*-wave changes as early as the fourth day of illness and as late as the fifty-seventh day with a return to normal in a period of 7 to 63 days. The data presented here are essentially in agreement with their observations.

Alstead[1] believes that the *T*-wave change is not indicative of serious myocardial damage and that the myocardium returns to normal with the return of the normal *T* wave. Nathanson,[5] however, studied 15 ambulatory cases of convalescent diphtheria and found 7 with abnormal *T* waves. Two of these patients died suddenly.

In our series death occurred in 3 of the 23 cases of *T*-wave change. One death was due to respiratory complications, 1 was probably due to a previous coronary disease and the third developed conduction changes.

Fatal terminations in this group of cases are not in agreement with our findings and we do not regard the *T*-wave changes to be of serious prognostic significance. Our experience may possibly be explained by the fact that we prescribed a period of complete inactivity for all the patients showing these alterations, until such time as the electrocardiogram had returned to normal.

Similar *T*-wave changes are noted in many febrile diseases such as scarlet fever, pneumonia, acute sinusitis, rheumatic fever and tonsillitis. They are frequently present in coronary disease and in patients who are adequately digitalized. With the exception of coronary disease the alterations are generally transitory and apparently the result of intoxication rather than structural damage to the myocardium. They appear to have little or no prognostic value. Our present studies of their occurrence in diphtheria would seem to show them to be similarly transient and largely without evil significance as to the patient's recovery.

Conduction Changes. The second group of electrocardiographic changes consisted of ventricular escape, prolonged *QRS* phase, intraventricular block and auriculoventricular (*A–V*) dissociation. Marked distortion of the *R–T* line, with depression or elevation in one or more leads, was of frequent occurrence. The cases showing intraventricular block presented electrocardiograms which closely approached those of a bundle branch block, but where there was doubt the records were interpreted as intraventricular block without further specific definition. Many cases showing *A–V* dissociation presented a terminal picture of ventricular tachycardia.

These conduction changes occurred in the more toxic cases, in the younger age group and, in sharp contrast to the changes in the *T* wave, proved to be of grave prognostic significance.

Of our series, 17 patients showed various types of conduction

FIG. 1

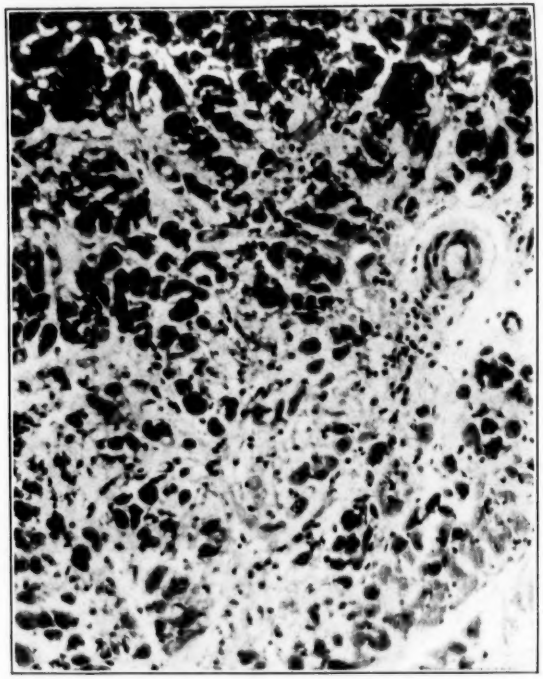


FIG. 2

FIG. 1.—Case 18. Female, aged 9. Death on ninth day of illness. Section from posterior papillary muscle. Note perivascular, patchy areas of intense leukocytic infiltration, associated with degenerative changes of muscle cells, generalized interstitial edema and congestion. See Fig. 6 for electrocardiogram.

FIG. 2.—Case 131. Female, aged 5. Death on tenth day of illness. Note acute myocardial degeneration with necrosis, leukocytic infiltration, hydropic and fatty changes.

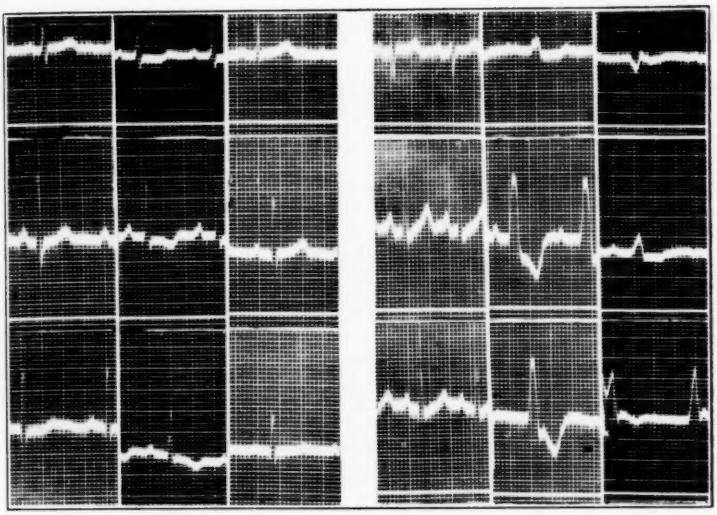
FIG. 3

FIG. 4

FIG. 3.—Case 133. Female, aged 6. Death on fourteenth day of illness. Section from interventricular septum. Note acute degeneration and necrosis of the muscle with beginning transition of the exudate from polynuclear to monocytic cells. There is an early fibrosis.

FIG. 4.—Case 17. Female, aged 19. Death on sixty-first day of illness. Section from posterior papillary muscle showing late interstitial fibrous myocarditis.

FIG. 5 FIG. 6

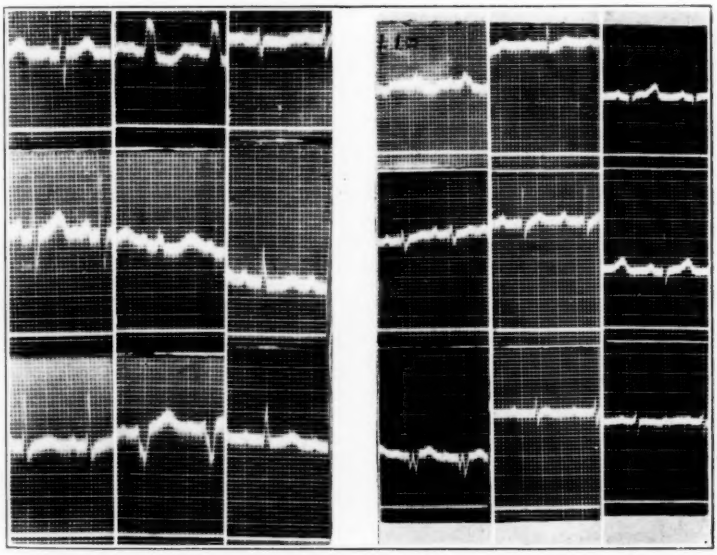


April 21 May 7 June 16 April 8 April 11 April 13

FIG. 5.—Case 5. Aged 28. Electrocardiograms on dates indicated. Note normal record followed by inversion of the *T* waves and subsequent return to normal. Patient recovered.

FIG. 6.—Case 18. Aged 9. Electrocardiograms on dates indicated. Note auriculoventricular dissociation in the second record. The excursions show a decreased amplitude in the final record. See Fig. 1. Patient died.

FIG. 7 FIG. 8



June 10 June 12 June 22 Feb. 23 Mar. 19 May 5

FIG. 7.—Case 41. Aged 5. Electrocardiograms on dates indicated. Note intraventricular block. The final record shows improvement. Patient recovered.

FIG. 8.—Case 10. Aged 3. Electrocardiograms on dates indicated. Note intraventricular block in the first record. The second record shows improvement but a first-degree block is present. The final record is normal. Patient recovered.

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defects. The earliest occurred on the fifth day and the latest on the thirteenth day of illness. The changes occurred on the eighth day, as an average for the group. The conduction defect was noted to persist for various periods of time, ranging from 1 to 13 days; average duration, 5 days.

Eleven of the 17 patients developed *A–V* dissociation. This complication was invariably fatal, which accounts for the short duration of life recorded for this group.

Four of the 17 patients recovered. One of them demonstrated ventricular escape, a mild form of conduction block. One showed splintering and a prolongation of the *QRS* phase. Two presented intraventricular block without *A–V* dissociation. Two of the patients who recovered subsequently also developed *T*-wave changes on the tenth and fifteenth day, respectively, after the onset of the conduction change (Table 2 and Case Histories).

TABLE 2.—TOXIC DIPHTHERIA CASES SHOWING ELECTROCARDIOGRAPHIC CHANGES IN THE CONDUCTION SYSTEM.

Case No.	Age.	Days from onset of illness to antitoxin.	Amount of antitoxin in 1000 units.	Days from onset to conduction change.	Days duration of change.	Days from onset to peripheral nerve paralysis.	Comment.
2	6	2	34	7	5	12	*A–V* dissociation; died.
4	2	4	35	9	12	15	*A–V* dissociation; autopsy.
10	3	2	30	10	7	...	Intraventricular block.*
16	4	6	38	9	4	27	Ventricular escape.*
17	19	3	82	†	†	32	Autopsy; 61-day illness.
18	9	2	40	6	3	...	*A–V* dissociation; autopsy.
33	4	5	30	6	10	15	*A–V* dissociation; died.
40	8	3	25	7	1	...	*A–V* dissociation; died.
41	5	4	30	8	13	32	Intraventricular block.*
52	6	4	30	8	9	10	*A–V* dissociation; autopsy.
81	2	4	20	5	1	...	*A–V* dissociation; died.
89	2	5	25	10	2	...	*A–V* dissociation; died.
107	2	3	35	10	5	20	Splintering *QRS*.*
131	5	5	30	7	3	...	*A–V* dissociation; autopsy.
132	3	6	38	9	1	...	*A–V* dissociation; autopsy.
133	6	10	30	13	2	10	Intraventricular block; autopsy.
134	12	5	35	11	5	11	*A–V* dissociation; died.
Average	5	4	34	8	5	18	

* Indicates recovery. † Records inadequate.

Age. The *T*-wave changes occurred in all age groups. They were more prevalent, however, in the electrocardiograms from patients between 18 and 57 years of age.

The conduction changes occurred in the younger age groups. They were more frequent in the electrocardiograms from patients between 2 and 6 years of age. The oldest patient showing this type of change was 19 years of age.

Antitoxin. Antitoxin was given to every patient included in this series, with the exception of 2. These entered late in the disease after the acute symptoms had subsided.

Contrary to what might have been anticipated some of the most marked electrocardiographic and pathologic changes occurred in patients who had received large doses of antitoxin as early as the second and third day of illness (Table 2, Cases 2, 10, 18; Figs. 1 and 6.)

The 17 patients showing conduction changes received an average of 34,000 units of antitoxin on the fourth day (average) of illness. The *T*-wave alteration group of 23 patients received an average of 42,000 units of antitoxin on the fifth day (average) of illness. The value of antitoxin cannot be deduced from the data presented as all of the toxic patients received this specific therapy. It is shown, however, that even the early administration of antitoxin does not always protect the patient suffering from a virulent infection.

Nerve Palsies. The incidence of peripheral nerve palsies in the group of patients showing electrocardiographic changes was so frequent that we include data on their occurrence. The nerve damage was manifested by nasal voice, nasal regurgitation, extra-ocular motor paralysis and severe weakness of the extremities.

The palsies occurred late in the illness and were, therefore, prevalent in the group surviving the acute diphtheria and they bore no definite causal relation to the cardiac phenomena other, perhaps, than their prevalence in the more toxic cases. Peripheral nerve palsies occurred in 17 of the 23 patients showing *T*-wave changes, an incidence of 74%. The earliest occurred on the eighth day of illness and the latest on the forty-fifth; average onset was on the twenty-third day.

Paralysis occurred in 10 of the 17 cases showing conduction changes, an incidence of 59%. Early mortalities account for this low figure. The earliest paralysis in this group occurred on the tenth day of illness and the latest on the thirty-second day. The average occurrence was on the eighteenth day as contrasted to the 23-day average for the group showing *T*-wave changes.

The high incidence of peripheral nerve palsies in these groups of patients is of important clinical significance. We find that many cases showing clinical nerve palsies have an accompanying toxic myocarditis. When conditions do not favor the routine use of electrocardiograms the development of a nerve palsy should suggest the immediate need for special care and every effort should be made to secure one or more electrocardiograms. Absolute bed rest and a prolonged convalescence should be demanded.

Clinical Manifestations of Toxic Myocarditis. Dyspnea, edema and enlarged, tender liver, so often encountered in myocardial failure, were rarely found in this group of young patients. The symptoms generally encountered were similar in many respects to those of coronary disease. Precordial pain, arrhythmias and shock were the predominant symptoms. Such circulatory failure as was observed was of the peripheral vasomotor type, similar to that seen

in other severe infectious diseases, or, at times, after surgical operations. The day to day cardiac ausculations proved to be a poor index to the appraisal of the myocardial function. *A–V* dissociation was often undetected until the electrocardiogram was obtained. Regular idioventricular rhythms of 80 to 90 per minute were not uncommon in the terminal phase of the diphtheria.

Marked arrhythmias, observed clinically, during the first 2 weeks of illness, caused us to suspect some major type of conduction block. This suspicion was generally confirmed by the electrocardiogram.

Marked *T*-wave alterations (diphasic and inverted) were always found during the convalescent stage of the disease. Routine clinical heart examinations revealed no signs of physical change with the possible exception of a tachycardia which was out of proportion to the temperature and clinical state of the patient. These facts lend support to the assumption that these changes are toxic rather than structural in nature.

The Pathologic Anatomy of Diphtheritic Myocarditis. It is our purpose to demonstrate the changes which occur in the myocardium and its conduction tracts in the fatal cases of diphtheritic myocarditis. We also attempt to correlate the anatomic changes with the alterations in the electrocardiographic tracings.

During the past 5 years we have had the opportunity of making complete anatomic studies in 100 cases of diphtheria. This represents approximately 54% of the total number of fatal cases, which in turn represents a mortality rate of 5.5% of 3220 diphtheria admissions at the Willard Parker Hospital during the same 5-year period. The correlated electrocardiographic and autopsy studies cover only a few selected cases on which serial electrocardiograms were obtained.

Broadly speaking, there is a rough parallelism between the degree of change in the conductivity as measured by the electrocardiograph, and the severity of the histologic changes in the myocardium. There is, however, a distinct lack of correlation between the electrocardiographic changes and demonstrable lesions in the conduction tracts.

We cannot outline the pathologic changes occurring in the myocardium in the *T*-wave alteration group. Death did not occur from this complication alone.

In the second group (7 cases) showing conduction changes electrocardiographically, there was a striking similarity in the tissue changes of the myocardium. In every instance there was more marked involvement of the contractile tissue than of the conduction tract.

Gross Lesions. It is a source of considerable surprise to the clinician when he attends an autopsy on one of his diphtheria cases, which has apparently died of myocardial failure, to have the pathologist find little or nothing to demonstrate in the heart grossly.

Yet that is the picture which is usually present. Certainly, in the majority of cases the changes are so minimal that, even to one familiar with the appearance of the heart in acute infectious disease, there is very little to suggest any actual impairment of heart function or structure. In the average case the myocardium, at most, seems to have lost a little of its tone; in the more striking cases, to be actually flabby in consistency. There may or may not be an associated right-sided dilatation. The endocardium rarely shows any gross abnormalities, being smooth and glistening. The valves, in conjunction with the dilatation, may be slightly stretched at times, but there is ordinarily no apparent valvulitis. Section of the musculature may on occasion suggest very minor fatty degeneration with some intimation of "tigroid" mottling.

In the 7 severe cases with which we are particularly concerned in this discussion the picture is more definite. There is a frank myocardial degeneration with softening of the musculature, in at least 2 instances, to a degree where the examining finger perforates the wall because of its extreme friability. Its color is quite definitely yellowish due to extensive fatty degeneration. In most of these cases, agonal or antemortem thrombi are found in the auricular appendage of the right side. In 1 instance (Case 52) a similar thrombosis on the interventricular septum was noted in the left ventricle. No grossly demonstrable changes involving the valves could be found.

The microscopic picture is likewise apt to be disappointing in the majority of cases in comparison with the clinical evidence of myocardial failure of which the patients seem to die. In this limited group of cases in which correlated studies of the electrocardiograms and the tissue changes were conducted, they appeared much more startling and severe. In our series we have progressive lesions ranging from practically *nil* to the most profound. These occurred in cases dying from the third day of the disease up to the sixty-first. No parallelism is noted in the time relationship in general, as we have cases dying in less than a week with very marked changes, and cases dying in the third and fourth week showing little or nothing histologically. On the other hand, in studying the pathologic changes of the entire 100 cases we find that the peak of the acute picture occurs somewhere between the tenth and the nineteenth day of illness. In those patients dying later than this the reparative features predominate.

In spite of the marked alteration in conductivity as shown by the electrocardiograms, the lesions of the conduction system are usually much less marked than the general myocardial damage. This has been demonstrated by silver stains of the nerve structure and by a few sections stained for glycogen by Best's carmine method.

The histologic features of diphtheritic myocarditis may be summed up very simply. First, there is a progressive interstitial edema and

congestion of the myocardium which may or may not be demonstrable in the conduction bundles, but which is usually especially notable in the auricular musculature, the papillary muscles of the right and left ventricles and the interventricular septum, in that order of frequency.

In the second place, we have a perivascular leukocytic infiltration of the stroma of increasing degree. This extends in the more severe cases throughout the musculature, but is apt to be patchy and irregular in its distribution. At times it becomes the predominant feature of the picture (Fig. 1). Associated with this interstitial myocarditis, we find varying degrees of degeneration of the muscle fibers: fatty and hydropic changes, swelling, loss of staining capacity, ultimately loss of striation, nuclear degeneration, usually of a karyolytic nature, and finally actual necrosis (Fig. 2). This parallels closely, of course, the gross changes already described. Whether these degenerative changes are the result of direct action of the toxin on the muscle fibers or follow indirectly as a result of the exudate, is difficult to ascertain, but arguing by analogy we believe the former to be true.

At this stage of the acute inflammatory process the reparative factors of transition of the exudate from the neutrophil to the mononuclear type and the new growth of fibroblasts sets in (Fig. 3). This is best demonstrated by special connective-tissue stains. For the most part, these severe myocardial changes have very closely paralleled the changes seen in the electrocardiographic record.

In the late picture, which we had the opportunity of seeing in 1 patient who died on the sixty-first day of illness, we find a typical diffuse fibrosis of the myocardium, comparable to the chronic interstitial fibrous myocarditis which we commonly associate with nutritional interference and resultant sclerotic changes in middle or later life (Fig. 4). We do not wish to overemphasize this picture, but we believe that individuals who have suffered from toxic diphtheria in childhood, especially with suggestive heart involvement, may represent potential cardiac invalids. The factor of safety in these hearts must be impaired by the scarring and functional hypertrophy of the remaining musculature.

Councilman, Mallory and Pearce[2] state that it is possible that diphtheria may lead to extensive formation of connective tissue and some of the cases of fibrous myocarditis may be due to it.

Only by following a series of such cases to middle life can an accurate determination of this relationship, if any, be made.

Case Histories. The following case histories illustrate the clinical course of patients demonstrating electrocardiographic changes, as described in the text. The pathologic findings are described in 2 of the 5 cases.

CASE 1.—*Changes in the T wave.* D. C. (No. 2169; 1932) (series Case 5), a male cab driver, aged 28, developed a sore throat on April 12. Local

physician incised infected area. He became progressively worse the next 2 days and was then given 10,000 units of diphtheria antitoxin intravenously on the fifth day of illness. Admitted to the hospital on April 17 where 20,000 units of antitoxin were given intramuscularly and a similar amount given intravenously.

Physical Examination (*Positive Findings*). Dysphagia and irritating cough were present. There was a thick gray membrane over the tonsils, uvula and soft palate. The neck showed bilateral confluent cervical adenitis. A throat culture showed Klebs-Loeffler bacilli.

Progress notes: April 18, Ekg. Rate, 108; *P–R*, 0.16 sec.; *QRS*, 0.06 sec. Sinus tachycardia. April 20, patient appeared toxic. Heart sounds normal, throat very sore. No edema or petechiæ. April 21 (see Ekg., Fig. 5). Rate, 74; *P–R*, 0.16 sec.; *QRS*, 0.07 sec.; normal record. April 23, gray membrane still covered throat. Ekg. as on April 21. April 24, throat improved, voice good. Heart, first sound at apex prolonged. Liver and spleen not enlarged. No edema. April 27, general condition much improved. No murmurs. No evidence of cardiac insufficiency. Ekg. showed initial inversion of T_2 and T_3. Rate, 65; *P–R*, 0.18 sec.; *QRS*, 0.06 sec. May 7, inversion of *T* wave in all leads. These changes continued until June 1. May 10, nasal voice first noted, regurgitation of food. Cough present. June 13, developed severe hyperesthesia of the extremities with a moderate degree of paralysis of the muscles of the feet, legs, hands and arms, Fig. 5. June 16, normal record. This had not entirely cleared on August 14, the day of discharge. June 16 (following year) Ekg. normal.

Case 2.—*Auriculoventricular dissociation.* E. M. (No. 1959; 1932) (series Case 18), female, aged 9, developed a sore throat and fever on April 5, admitted to hospital April 7 acutely ill, when 20,000 units of diphtheria antitoxin were given intramuscularly and an equal amount given intravenously.

Physical Examination (*Positive Findings*). Toxic in appearance. Thick nasal discharge. Extensive dirty membrane covering the tonsils and soft palate. Marked cervical adenopathy typical of a "bullneck." Nose and throat cultures showed Klebs-Loeffler bacilli.

Progress notes: April 8, Ekg. Rate, 103; *P–R*, 0.14 sec.; *QRS*, 0.05 sec.; right axis deviation (Fig. 6); temperature, 101° F. Very toxic—general condition poor. April 9, heart sounds of poor muscular quality. Trace of albumin in urine. April 10, condition much worse, poor renal output. Pitting edema over tibiæ. April 11, Ekg. Auriculoventricular dissociation. Auricular rate, 105; ventricular, 86. Pulse regular, between 80 and 90. April 12, glucose infusion given. Marked pallor present. Ekg. as April 11, except ventricular rate, 56. April 13, very toxic. Heart sounds not remarkable. Ekg. except auricular rate, 122; ventricular rate, 75. The excursions showed a decreased electrical potential as compared with previous records. Patient suddenly vomited, developed generalized spasm, heart sounds became distant with rate of about 200 to 220 per minute. This continued for 10 to 15 minutes and the patient died. A terminal Ekg. was not obtained.

Summary of Case 2 (autopsy findings). The heart is enlarged. There are no valvular defects, but there is a definite, grossly demonstrable myocarditis, with several subintimal petechiæ. There is fatty mottling and loss of muscle tone of the myocardium. Microscopically, there is a very marked, acute myocarditis, characterized by interstitial edema, congestion, considerable mononuclear cellular infiltration, and beginning fibrosis. The muscle fibers show fragmentation, fatty hydropic degeneration, and in many places hyalinization is present (Fig. 1). The lesions of the heart seem to be fairly uniform and involve the entire musculature. There is no particular predilection for the conduction system and the bundle of His

shows rather less extensive changes than does the rest of the musculature. The lungs show a terminal hemorrhagic bronchopneumonia, limited to the right side. The abdominal viscera present no pathologic changes beyond moderate toxic, degenerative changes.

CASE 3.—*Intraventricular block.* E. B. (No. 4125; 1933) (series Case 41), female, aged 5, developed a nasal discharge, cervical adenitis and vomited on June 5. Admitted to hospital on June 8 where 15,000 units of diphtheria antitoxin were given intramuscularly and an equal amount given intravenously.

Physical Examination (*Positive Findings*). Very toxic. Marked bilateral adenopathy ("bullneck"). Tonsils, uvula and soft palate were covered with a gray membrane. Nose and throat cultures showed Klebs-Loeffler bacillus.

Progress notes: June 9, Ekg. Rate, 96; *P–R*, 0.16 sec.; *QRS*, 0.06 sec. Right axis deviation. No murmurs. June 10, Ekg. as June 9 (Fig. 7). June 12, short systolic apical murmur developed. Ekg. Rate, 98; *P–R*, 0.16 sec.; *QRS*, 0.11 sec. Depressed *S–T*. Diagnosis: intraventricular block. This block continued with a decreasing *QRS* interval until June 22. Ekg. showed splintering of *QRS* in all three leads. June 24, Ekg. returned to normal. Rate, 95; *P–R*, 0.18 sec.; *QRS*, 0.05 sec. Heart sounds of good quality. General condition much improved. July 7, developed nasal voice; July 27, voice clear. Aug. 1, discharged cured.

CASE 4.—*Intraventricular and first-degree block.* H. F. (No. 733; 1932) (series Case 10), male, aged 3. February 10, developed sore throat and vomited. Admitted to hospital February 12, where 20,000 units of diphtheria antitoxin were administered intravenously (10,000 units next day).

Physical Examination (*Positive Findings*). Acutely ill. Serosanguinous nasal discharge, gray membrane on both tonsils and soft palate. Cervical nodes enlarged. Nose and throat cultures showed Klebs-Loeffler bacilli.

Progress notes: February 23, Ekg. Rate, 120; *P–R*, 0.16 sec.; *QRS*, 0.12 sec. Slurred *QRS* in Lead 1 (Fig. 8). Splintered *QRS* in Leads 2 and 3. Intraventricular block. February 27, signs of cardiac decompensation. Liver border to umbilicus, slight pre-tibial edema. Frequent premature ventricular contractions. Digitalized. March 1, Ekg. Rate, 112; *P–R*, 0.16 sec.; *QRS*, 0.06 sec. Low potential throughout. Slight splintering *QRS* in all leads. March 2, vomited. Heart sounds of poor quality. No liver tenderness or enlargement. No murmurs. March 5, Ekg. Rate, 94; *P–R*, 0.2 sec., *QRS*, 0.06 sec. Low potential throughout. March 10 to 14, vomited daily. March 14, otitis media. March 19, Ekg. Rate, 92; *P–R*, 0.23 sec.; *QRS*, 0.06 sec. First-degree block. March 20, slight pretibial edema. March 24, Ekg. Rate, 94; *P–R*, 0.18 to 0.2 sec.; *QRS*, 0.06 sec. A tendency to migratory pacemaker. March 26, systolic apical murmur developed during the last 3 days. Rhythm regular. Heart not enlarged. March 28, Ekg. Rate, 103; *P–R*, 0.14 sec.; *QRS*, 0.04 sec. Normal record. April 2, murmur still present. April 5, patient developed scarlet fever. April 25, developed measles. During the course of these two cross-infections the contour of the electrocardiogram did not change. May 5, patient discharged cured, Ekg. normal.

CASE 5.—*Fibrosis of myocardium.* A. M. (No. 258; 1932) (series Case 17), female, aged 19, developed a sore throat and vomited on January 15. Admitted to hospital January 18 with severe sore throat and swelling of neck. Diphtheria antitoxin (22,000 units) were administered intravenously just before admission. On admission, 15,000 units were given intravenously and a similar amount given intramuscularly. January 19, 15,000 units intravenously.

Physical Examination (*Positive Findings*). Heavy adherent gray membrane of soft palate extending to midportion of hard palate covering the

fauces and obstructing the larynx. Cervical adenitis ("bullneck"). Nose and throat cultures showed Klebs-Loeffler bacilli.

Progress notes: January 20, worse. Breathing difficult from throat edema. Edema of scalp and "bullneck." Petechiæ over body. Diphtheria antitoxin (15,000 units) given intravenously. January 21, prognosis probably fatal in 48 hours. B.P., 110/84. January 23, some improvement. No heart murmurs. January 24, 20 cc. 50% glucose given intravenously and 10 units of insulin. January 25, no edema. No signs of heart failure. January 27, severe precordial pain; vomited. February 1, no edema, improved. February 12, condition very good. February 17, nasal voice (34th day illness). February 24, vomited, difficult swallowing. March 1, first Ekg. taken (first case studied and few tracings obtained). Rate, 84; *P–R*, 0.15 sec.; *QRS*, 0.06 sec.; slurred *QRS* in Leads 2 and 3. Diphasic T_2, negative T_3. March 6, Ekg. as on March 1. Vomited. March 11, occasional premature ventricular contraction. March 12, Ekg. as on March 1. March 14, heart sounds of good quality. No murmurs. March 20, vomited, and temperature progressed to 102° F. No edema. Patient suddenly became restless with severe coughing. Appeared very toxic. Examination showed heart to the right with apex beat at left sternal border. Resonance over entire right lung field with marked diminution of breath sounds. Diagnosis: massive collapse of right lung. Sudden death (61st day of illness).

Summary of Case 5 (autopsy findings). Terminal diffuse bilateral bronchopneumonia superimposed on a collapse of the right lung.

The heart is moderately dilated; myocardium, pale and flabby. There are no valvular or endocardial lesions. Sections microscopically through the heart show a diffuse generalized myocarditis in the stage of repair. The most striking finding is a very extensive fibrosis. This is relatively early, as evidenced by the large number of nuclei and the comparatively small amount of collagen. There is still considerable cellular infiltration, chiefly about the blood-vessels, but extending into the interstitial tissue. The cells are mostly of the monocytic series, including lymphocytes, plasma cells and large mononuclear phagocytes. No greater involvement of the bundle of His is noted than of the musculature in general (Fig. 4). The remainder of the visceral lesions are of relatively slight significance. There is an associated toxic hepatitis, splenitis and toxic degeneration of the renal tubular epithelium.

Summary and Conclusions. 1. Serial electrocardiograms were made on 140 patients showing evidence of toxic diphtheria; 28 of these showed changes in the contour of the electrocardiograms.

2. The electrocardiographic changes were divisible into two groups comprising *a*, alterations in the *T* wave, and *b*, alterations in the conduction system.

3. Twenty-three patients showed the *T*-wave changes occurring between the fifth and thirty-ninth day of illness. A majority of the changes occurred between the eighth and fifteenth day of illness.

4. Seventeen patients showed conduction changes between the fifth and thirteenth day of illness; 11 of these patients developed *A–V* dissociation. This complication invariably proved fatal.

5. Fourteen patients, showing electrocardiographic changes, died of toxic diphtheria; 7 of these received large doses of diphtheria antitoxin on or before the fourth day of illness. Early administration of antitoxin did not save this group of patients.

6. Peripheral nerve palsies occurred in 65% of the patients

presenting electrocardiographic changes. The paralysis apparently bore no causal relationship to the cardiac phenomena.

7. There was a rough parallelism between the conductivity as shown by the electrocardiogram and the microscopic changes in the myocardium as demonstrated in the 7 cases of this series that were autopsied.

8. The essential histologic changes in the myocardium due to toxic diphtheria are shown to be progressively, edema, congestion, cellular infiltration, degenerative changes in the muscle fibers and ultimate fibrosis.

9. These lesions, found at autopsy, suggest that diphtheria may be one of the causes of chronic fibrous myocarditis in patients who survive the more toxic state.

10. The electrocardiographic findings constitute an important guide in the treatment of diphtheria. Complete inactivity is recommended for those showing abnormal electrocardiograms until the electrocardiogram has had ample opportunity to return to normal.

The technical work (electrocardiographic) for this paper was preformed by Miss Marion Frank, R. N.

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THE EFFECTS OF CAROTENEMIA ON THE FUNCTION OF THE THYROID AND THE LIVER.*†

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Avitaminosis has received much attention in medical literature in the past few years, but few reports have appeared on hypervitaminosis. It has been maintained§ that carotene is non-toxic

* Supported, in part, by the Christine Breon Fund for Medical Research.

† With the technical assistance of Mrs. Media Orem Dahl, A.B., Mrs. T. B. Leake, A. B., and Miss Mabel Stockholm, A.B.

‡ Now with the Council of Medical Education and Hospitals of the American Medical Association.

§ The Non-toxicity of Carotene. Abstracts prepared by the Research Department of S. M. A. Corporation, Cleveland, Ohio.

and may be used therapeutically in large amounts. While it is not likely that the excessive use of therapeutic preparations of carotene will produce carotenemia, we have seen carotenemia in patients who have been on abnormal diets which contained large amounts of vitamin A and its precursors. We are reporting in this study certain untoward effects which have followed the use of such diets and which should be recognized, especially disturbances of the thyroid and the liver.

There is considerable laboratory and clinical evidence suggesting a possible antagonism between carotene and thyroxin. In 1932, Euler[4a] fed rats a diet deficient in vitamin A but which contained adequate amounts of vitamins B, C and D. Carotene and thyroxin were then administered to one group, thyroxin alone to another and carotene alone to a third. The first group gained an average of 1 gm. per day in body weight; the second lost 9 gm. a day; while the animals receiving carotene without thyroxin gained 11 gm. daily. The administration of carotene with thyroxin seemed to compensate for the loss in body weight due to the administration of thyroxin alone. In another experiment on rats, Euler[4b] fed a diet deficient in vitamin A and found that a partial inactivation of thyroxin occurred, possibly due to fixation or binding of the thyroxin by the carotene.

Euler refers to the work of Abelin[1a] who confirmed his observations. He used guinea pigs kept on a diet deficient in vitamin A. Abelin also found that when colloidal carotene was mixed with thyroxin and allowed to stand in a stream of air in an incubator, in 4 or 5 instances the thyroxin content of the mixture decreased approximately 22.5%. The author implies that no change in thyroxin content occurred using thyroxin alone.

Parhon and Werner[9] gave 500 units of thyrotropic hormone to ducks over a period of 5 days and found the mean blood carotene content to be 0.453 mg. per 100 cc., whereas controls had 0.664 mg. per 100 cc. The ducks given the hormone alone lost 6.33 gm. per 100 gm. during the period of experimentation.

Wendt[11] reported low serum carotene and vitamin A in Basedow's disease, but following treatment with iodine or by thyroidectomy the vitamin A content of the serum was increased. When he gave large doses of vitamin A to 5 patients with hyperthyroidism who had previously responded poorly to other methods of treatment, 3 gained weight, had decreased metabolic rates, and showed subjective and objective improvement. Abelin[1b] also suggested that vitamin A be used as an adjunct to iodine in the treatment of hyperthyroidism. He believed, however, that a definite evaluation of this procedure was not possible.

Laubmann[6] found that when rats were given toxic amounts of vitamin A they showed slight parenchymal changes in the liver and the spleen, and died as a result of glomerulonephritis. It is

difficult[3] to cause hypervitaminosis A in animals by feeding excessive amounts of carotene, since the liver of rats absorbs but a small amount of carotene as compared with absorption of vitamin A. Animals and men are known to store large quantities of vitamin A in the liver, so that as much as 95% of the total amount distributed in the body may be recovered from this organ. Ocana[7] noted an increased content of fat in the cells of the livers of rats given large doses of carotene. This investigator fed toxic amounts of extracts of carrots to rats and was able to produce lipoidosis of the cells of the liver. It is conceivable, therefore, that excessive carotene or vitamin A in the liver might cause some impairment of hepatic function.

Several workers have reported an increased cholesterol of the blood serum of rats and men in hypervitaminosis A.[5] Lasch[5] believed this was due to a direct action of the vitamin on the liver. Pasternak and Page[10] studied the action of thyroxin on lipoid metabolism and found that the iodine number was increased 50% in the muscles of thyroxin-injected rabbits, but there was no increase in the cholesterol content. The fat and fatty acids in the muscle were decreased. There was little change in these constituents in the liver, brain and blood except that the liver had an increased cholesterol in the rabbits treated with thyroxin. Parhon and Ornstein[8] reported that daily doses of 1 mg. of thyroxin given to patients for 2 to 3 days decreased their cholesterolemia as well as the fatty acids and total lipids in the blood.

These observations suggested to us the hypothesis that patients with carotenemia might exhibit clinical evidence of hypothyroidism or hepatic insufficiency.

We have reviewed 13 patients with carotenemia,* in 9 of whom functional tests of the thyroid and liver were made. All of the patients were adults, aged 19 to 65 years; 7 were males. Nine lived in rural districts of California, and of these 5 were farm residents. The diagnosis of carotenemia was made by observing the yellowish discoloration of the skin, which was noted in the palms of the hands and the soles of the feet. The scleræ were not discolored nor did other areas of the skin show perceptible changes in color. Since Baur[2] has shown that carotene in solution was changed chemically on exposure to light and air, it seems likely that this photo-oxidative effect is more pronounced on surfaces of the body other than the palms and soles. The clinical diagnosis was substantiated in all cases by the appearance of carotenoid coloring of petroleum ether when shaken with a specimen of the blood serum from which protein had been precipitated by alcohol. No quantitative estimate of carotene was attempted.

* Five patients included by courtesy of Dr. Alfred C. Reed; 2 by courtesy of Dr. Wm. J. Kerr; and 1 by courtesy of Dr. I. C. Schumacher.

TABLE 1.—SIGNIFICANT FINDINGS IN 9 PATIENTS WITH CAROTENEMIA.

Initials and case No.*	Diagnoses other than carotenemia.	Approximate weight on entry in kg.	Duration of abnormal diet.	Estimated loss of weight in kg.	Basal metabolic rate, %.	Rose-Bengal test,† % dye remaining in blood after:		Plasma cholesterol, mg. %.	Total blood lipids,‡ gm. %.	Total phenolsulphone phthalein excreted in 2 hrs., %.	Other observations including complaints.
						8 min.	16 min.				
M. A. 1	Bursitis; hepatic insufficiency	60	1 yr.	3.6	−10.7	75	44	208	1.66	...	Lived on farm; dieted for "constipation."
V. H. 2	Fibroid tumor of uterus; hypothyroidism	62	1 mo.	3.2	−18.0	62	30	197	2.00	...	Urban resident; dieted for "constipation."
O. J. 4	Hypothyroidism; hepatic insufficiency	63	2 yrs.	6.1	−40.7§	62	44	238	...	...	Lived on farm; complained of epigastric pain, bloating.
J. H. 5	Hepatic insufficiency; chronic prostatitis	70	Not known	0	...	84	52	...	...	65	Farmer; suffered nocturia, complained of bloating.
K. N. 6	Hepatic insufficiency; anxiety neurosis	74	2 mos.	3.2	...	80	61	...	...	100	Farmer (tractor mechanic); had epigastric pain, bloating.
W. N. 7	Chronic cholecystitis; without hepatic involvement	65	6 mos.	18.0	...	42	36	...	...	...	Rural resident; complained of epigastric pain.
J. S. 8	Hypothyroidism; malnutrition	73	4 yrs.	20.9	−22.0	52	31	...	...	...	Farmer; suffered pain in epigastrium.
C. R. 12	Chronic cholecystitis; cholelithiasis; cirrhosis of liver; hypothyroidism	56	2 yrs.	5.6	−25.9	...	...	204	1.70	47	Rural resident; had epigastric pain, "constipation."
C. M. 13	Trigeminal neuralgia; arteriosclerosis; hypothyroidism	62	10 yrs.	6.0	−20.4	50	15¶	252	1.80	58	Rural resident; had upper lip pain and "neuritis."

* Cases 3, 9, 10 and 11 are not included because of incomplete laboratory data.
† Percentage remaining in blood normally is 55 ± % at 8 minutes and 35 ± % at 16 minutes.
‡ Method of W. R. Bloor (J. Biol. Chem., **23**, 317, 1915). (Normal values, 0.67 to 0.72 gm. %.)
§ On discharge from hospital the blood serum showed no carotenoid discoloration and the basal metabolic rate was −19%.
¶ Courtesy of Dr. Jesse Carr.
... No test done.

The carotenemia was due in each case to the substitution of vegetables, fruit and milk rich in carotene for other foods. It was apparently self-induced in 10 cases; 3 patients developed carotenemia on diets prescribed for peptic ulcer, diabetes mellitus and asthma, respectively. None of these patients sought medical care because of carotenemia. Table 1 summarizes the significant findings in 9 patients.

The most consistent finding in this group is the loss of weight during the period of dieting. Five (Cases 2, 4, 8, 12, 13) of the 7 patients in which the thyroid status was studied had clinical and laboratory evidence of hypothyroidism. The sixth patient (Case 1) had impaired hepatic function, a metabolic rate of -10.7%, but was not thought to be hypothyroid. In the seventh patient (Case 10), the determination of the basal metabolic rate was reported to be unsatisfactory. Only 1 patient with hypothyroidism had hepatic insufficiency. The 2 remaining individuals with hepatic dysfunction had no obvious cause other than carotenemia for this condition. Hepatomegaly occurred in 4 patients (Cases 1, 2, 6, 12) and tenderness in the right upper abdominal quadrant in 2. Rose-Bengal tests for hepatic function were made on 8 patients (serum concencentration of the dye was determined colorimetrically in specimens of venous blood withdrawn 8 and 16 minutes after 100 mg. of the dye had been injected intravenously). The blood lipids were significantly elevated in the 4 cases examined.

Icteric indices were elevated, presumably because of the carotenoid coloring of the blood serum. Van den Bergh tests were normal in the 6 patients tested. Routine blood, urine and stool examinations were normal except in Case 4 who had secondary anemia, and in Case 5 who had prostatitis. Blood Wassermann tests in 12 patients were negative. There were no other significant objective or subjective findings relating to carotenemia in these patients.

We may say, then, on the basis of our findings in 5 patients with carotenemia, that there is evidence indicating that an antagonism exists between carotene and thyroxin. Of the 5 patients with evidence of hypothyroidism, 1 had hepatic insufficiency; 3 others had laboratory and clinical evidence of hepatic dysfunction. It seems possible that carotenemia may have contributed to the dysfunction of the thyroid and of the liver in these individuals. Unfortunately, we have no data as to the status of these patients before the appearance of carotenemia. It is possible that in Case 8, malnutrition may have contributed toward the lowered metabolic rate.

Conclusions. Carotenemia and its complications deserve more serious consideration as a symptom complex or as a disease entity. Eight of 9 patients with carotenemia on whom functional tests were made had disturbances of the thyroid or liver or both. These could be best explained on the basis of Euler's suggestion that carotene

antagonizes thyroxin, and on the evidence presented that carotene damages the liver.

NOTE.—Dr. Wm. J. Kerr suggests that perhaps a primary injury of the liver may prevent the conversion of carotene and its storage as vitamin A. Thus from the evidence presented, hepatic insufficiency may be regarded as the cause rather than the effect of oversupply of vitamin A in the diet. Perhaps these patients had previous hyperactivity of the thyroid over long periods, and this resulted in hepatic injury. Following hyperplasia of the thyroid or "thyroiditis," hypothyroidism may occur. If vitamin A and thyroid function are antagonistic, a deficiency of the liver may prevent both the extraction of vitamin A and conversion of carotene which, being unchanged, is not available for storage as vitamin A and appears in the blood and tissues in the clinical state of carotenemia.

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BLOOD SUGAR DURING LABOR, AT DELIVERY AND POST-PARTUM, WITH OBSERVATIONS ON NEWBORNS.

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FROM time to time, various investigators have studied the blood-sugar values during pregnancy, at delivery and during the puerperium. Most of this work has been a matter of record for a number of years, during which period our knowledge of glucose metabolism has advanced rapidly and obstetrical practices have undergone great changes. Moreover, chemical techniques have been developed which enable us to follow with frequency and safety the blood-sugar values of newborns. In the light of this newer knowledge, it has seemed desirable to review the obstetrical blood-sugar values and contribute the blood-sugar values of newborns.

As the first step in our studies of glucose metabolism in obstetrics, we wish to report a series of blood-sugar values for normal mothers and infants at delivery. This work has been supplemented by blood-sugar determinations during the first stage of labor, during the puerperium, and on the infants of these mothers during the

first 3 days of life. We have to a certain extent been guided by Morriss,[2] feeling that his work on this subject represents the most complete study of blood sugar in pregnant women at delivery, and that this approach provides a means of comparison which renders our work more valuable from an obstetrical and laboratory point of view.

In 1917, at the time of Morriss' study, the most widely used test for blood sugar was that of Lewis and Benedict, in which picric acid is converted to sodium picramate, and the resulting orange-red color read against a standard similarly treated. At the present time, colorimetric blood-sugar methods may be classified as macro, requiring amounts of blood necessitating venipuncture, and micro, requiring small amounts of capillary blood obtainable from skin punctures. It is extremely important to specify the method used for determination of blood sugar, and to give the corresponding normal range.

TABLE 1.—NORMAL VALUES OBTAINED BY COLORIMETRIC METHODS FOR BLOOD SUGAR.

Name of test.	Normal range, mg.	Type of test.
Folin-Wu, 1929	90–120	Macro; copper reduction.
Folin-Wu, modified	90–120	Micro; 0.1 cc. of blood.
Benedict, 1931	70–90	Macro; copper reduction.
Benedict, 1931, micro	70–90	Micro; 0.1 cc. of blood.
Lewis Benedict, 1913	90–120	Macro; picric to pricramate.
Folin-Malmros, 1929	70–90	Micro; ferricyanide to ferrocyanide, 0.1 cc.
Jeghers-Myers modification of Folin-Malmros, 1930	70–90	Micro; 0.025 cc. of blood.

Former workers have used macro methods, necessitating the use of blood from venipuncture of the mother and from the cord of the infant, thus limiting the number of determinations. The work of Révész and Turolt,[4] who found that the blood sugar of the umbilical arteries was over 10% higher than that of the umbilical vein, indicates that cord blood is a mixture of uncertain composition so far as blood sugar is concerned. We feel that in using micro methods we are comparing the same type of blood from infant and mother, and enjoy an advantage in ease and safety of collection.

The method selected for determining the blood sugar in the present study was the Jeghers-Myers modification of the Folin-Malmros micro test, which is based upon the reduction of alkaline ferricyanide to ferrocyanide and subsequent color production with an acid ferric reagent. By running a series of standards equivalent to 70, 105, 140 and 175 mg. of glucose per 100 cc., accurate readings can be obtained over the range anticipated in this work. Precipitation of proteins directly from the capillary pipette used for collection prevents glycolysis. The use of this method makes it possible to run duplicates on the filtrate obtained from 50 cmm. of blood.

Unlike copper reduction tests ferricyanide reduction tests are not affected by auto-oxidation, and are therefore less susceptible to technical errors.

In our hands the modified Folin-Malmros test has given, in the great majority of determinations, an accuracy of within 3% on duplicates from the same filtrate. Several times the method has been checked by obtaining duplicate samples from the same skin puncture; such duplicates yielded results within 5%. To insure uniformity of technique, all blood samples were collected and all tests run by the same person. Workers familiar with colorimetric micro methods will realize that such tests are susceptible to an error of at least 3%,[3] and that the figures presented are possible only with great standardization of procedure. Table 2 summarizes the data on determinations made during the study.

TABLE 2.—NUMBER AND PERCENTAGE OF DETERMINATIONS ON MOTHERS AND INFANTS.

	Maternal.		Infant.		Total.	
Classification.	No.	%	No.	%	No.	%
No duplicate secured . .	31	11.4	81	29.6	112	20.6
Duplicates did not check .	35	12.5	27	10.0	62	11.4
Duplicates check to 3% .	205	76.1	165	60.4	370	68.0
Total determinations . .	271	..	273	..	544	

Of the 544 determinations run during the study, 432 (79.4%) were in duplicate; 62 (14.3%) tests run in duplicate did not check to 3%, usually for one of two reasons: (1) Low values difficult to compare due to light colors, (2) checks to 5% but not to 3%.

Following rigorous technique (cap and mask, preliminary scrub, sterile gown and gloves) samples of blood were collected from the heel of newborn infants as soon after delivery as respiration was well established, and at various intervals after birth. Because of this care, no complications were encountered during the study. Two infants developed chemical conjunctivitis which rapidly disappeared, and 1 infant showed signs of hemorrhagic disease of the newborn several hours after tests were taken. This infant later bled profusely from a slight self-inflicted scratch and from the umbilical stump, but recovered following a transfusion of whole blood.

Samples of the mother's blood were collected from the finger of the patient at the moment of delivery. No complications were observed in this group.

In view of the great difference in present-day obstetrical practices, we summarize here the care received by our cases during labor. Forty of the 50 cases were primiparæ, and received about the same type of care. The majority of them entered the hospital during the early stages of labor and were given morphine sulphate, $\frac{1}{6}$ grain, and scopolamine hydrobromide, $\frac{1}{150}$ grain, hypodermically, when contractions became uncomfortable. In 45 minutes this was followed by scopolamine, $\frac{1}{200}$ grain; and by a third dose of scopolamine,

$\frac{1}{400}$ grain, after another 45-minute period. Following this, patients received scopolamine, $\frac{1}{400}$ grain, at intervals of $1\frac{1}{2}$ hours until within 3 hours of expected delivery. Only occasionally was sodium amytal per rectum used, this drug being reserved for use in the multiparæ. With a few exceptions in which nitrous oxide was used, ether analgesia was given by the Gwathmey paper cone in the latter hours of the first and during the second stage of labor. All patients were anesthetized and the delivery, excluding breeches and versions, was effected by low forceps of the Tucker-McLane type, and was, in the great majority of cases, accompanied by mediolateral episiotomy. Pituitary extract (1 cc.) was given immediately after delivery, and ergotrate ($\frac{1}{320}$ grain) following the expression of the placenta. Mucus was removed from the baby's pharynx by catheter, and in some instances when respirations were shallow carbon dioxide, 5%, and oxygen, 95%, under pressure, were administered intermittently at normal respiratory rate.

In the multiparous group, the drug of choice was sodium amytal, 9 to 15 grains, per rectum, accompanied by ether analgesia in the latter part of labor, until delivery was possible. In this group Tucker-McLane forceps were also used in delivery of the head, but episiotomy was seldom necessary. The care of the baby was the same as in the primiparous group.

Our cases are unevenly distributed with regard to parity, since it is the policy at this hospital to deliver primiparæ in the hospital and most multiparæ in the home on the district service.

Table 3 lists the data on maternal blood sugar, infant blood sugar, total duration of labor, duration of the second stage of labor, duration of analgesia plus anesthesia prior to delivery, and notes on anesthetic, medication and type of delivery. Statistically, the proper treatment of this material to ascertain the interdependence of blood sugar and the various other factors has seemed to be the calculation of the Pearson rank-order coefficient of correlation.* These coefficients are incorporated in the table.

The mean blood sugars for mothers and infants are also shown in the table. The mean blood sugar for 10 multiparæ is 128.2, and for their infants, 101.7, all the determinations on infants being lower than those of their respective mothers. The mean maternal blood sugar for 40 primiparæ is 123.7, for their infants, 104.1. Three of the 37 infants had blood sugars slightly higher than those of their mothers, and the infant showing the greatest difference was 56.2 mg. less than his mother. The mean maternal blood sugar for the 50 mothers was 124.6, and for the 47 infants, 103.6. Fifty per cent

* Coefficient of correlation is a statistical device for expressing, in a single figure, the relationship between two series of data. Perfect direct relationship is $+1$, while perfect inverse relationship is -1; in practice the coefficient is generally less than 1, since relationships are seldom perfect. The Pearson formula is $p = 1 - \frac{6\,\Sigma d^2}{n(n^2 - 1)}$.

TABLE 3.—DATA ON ROUTINE CASES.

Case No.	Blood sugar, mg./100 cc.		Labor, hours.		Anesthesia and analgesia.		Medication.	Notes.
	Mother.	Baby.	Total.	Second stage.	Type.	Hours.		
								Multiparæ.
51	96.8	86.6	11:10	:44	GOE*	:25	M&S, amyt.	Low forceps.
87	100.2	83.8	10:26	:56	GO†	4:55	Amyt.	Low forceps.
90	110.0	78.0	7:57	2:27	E‡	3:25	Amyt.	Mid-forceps, mod. Scanzoni.
56	116.8	78.2	20:10	1:10	E	1:30	Amyt.	Spontaneous.
36	121.8	115.4	9:57	:57	E	:40	M&S, amyt.	Low forceps.
65	128.0	116.1	24:37	:50	GOE	2:20	M&S	High forceps, mod. Scanzoni.
91	130.5	80.0	24:56	:56	E	5:20	Amyt.	Low forceps.
54	141.0	88.6	9:27	1:27	E	6:15	Amyt.	Low mid-forceps.
7	149.4	135.7	11:10	:44	E	6:30	Amyt.	Podalic version, breech extr.
29	187.4	154.4	22:15	3:15	E	1:40	M&S	Mid-forceps, axis traction, modified Scanzoni.
Mean	128.2	101.7	15:13	1:21				
								Primiparæ.
73	98.4	81.1	11:32	:32	E	1:30	Amyt.	Podalic version, breech extr.
45	102.5	84.0	..	..	E	:20	None	Spontaneous.
83	102.5	96.5	12:09	1:24	E	3:00	M&S	Low mid-forceps.
38	103.0	95.7	43:23	2:23	E	2:30	M&S	Low forceps.
46	104.5	74.1	12:08	2:08	E	3:15	M&S, amyt.	Podalic version, breech extr., epis.
4	104.7	..	33:50	1:50	E	1:45	M&S	Low forceps, episiotomy.
22	105.3	87.7	12:50	2:05	E	1:35	M&S	Low mid-forceps.
24	106.6	110.0	12:20	:35	E	2:25	M&S	Breech.
26	107.4	86.4	13:40	:35	E	3:10	M&S	Low mid-forceps.
63	107.5	117.6	8:27	:57	E	1:05	M&S, amyt.	Low forceps, episiotomy.
37	108.8	95.3	18:06	:51	E	2:45	M&S	Low mid-forceps, modified Scanzoni, episiotomy.
64	110.2	93.8	15:25	1:55	E	:35	Amyt.	Low mid-forceps, episiotomy
75	112.6	97.4	29:44	1:44	E	2:45	M&S, amyt.	Low mid-forceps, mod. Scanzoni.
58	114.8	97.4	13:32	1:07	E	2:05	M&S	Low forceps.
61	117.7	110.9	14:15	1:30	E	4:20	M&S	Low mid-forceps.
49	118.0	84.7	13:55	2:10	E	2:55	M&S	Low forceps.
43	118.0	..	7:50	1:50	E	2:40	M&S	Low mid-forceps.
71	120.7	119.3	15:26	1:06	E	1:15	M&S	Low forceps, episiotomy.
69	121.8	99.5	14:20	:50	E	1:10	M&S	Low mid-forceps, mod. Scanzoni.
13	122.5	..	10:36	:51	GO	1:05	M&S	Low forceps, 6 weeks premature.
12	123.3	99.0	8:55	:55	E	2:10	Amyt.	Low mid-forceps.
47	124.2	117.0	34:30	1:30	E	1:30	M&S, amyt.	Low forceps.
41	124.6	86.4	7:47	2:17	E	2:15	M&S	Low forceps.
70	124.8	102.7	7:07	:52	E	2:15	Amyt.	Low forceps, mod. Scanzoni.
28	124.8	114.7	14:47	5:02	E	2:25	M&S, amyt.	Podalic version, breech extr.
84	127.2	91.3	12:45	1:00	E	3:08	Amyt.	Low mid-forceps.
68	127.5	106.1	14:08	2:08	E	1:25	M&S	Low forceps, episiotomy.
40	129.2	108.2	12:55	2:10	E	2:10	M&S	Low forceps.
74	130.4	117.4	10:55	2:45	E	2:35	M&S, amyt.	Low forceps, episiotomy.
66	131.4	105.0	9:55	:55	GOE	:20	M&S	Analgesia only; spontaneous.
80	133.9	109.3	11:28	3:28	E	3:00	M&S	Mid-forceps.
21	135.5	140.0	12:05	1:35	E	2:20	M&S	Low forceps.
86	136.9	106.1	13:00	1:30	E	2:00	M&S	Low mid-forceps, mod. Scanzoni.
30	137.3	104.8	11:08	1:08	E	6:25	M&S, amyt.	Breech extraction.
34	137.7	122.2	11:23	2:23	E	3:30	M&S	Low forceps.
35	139.5	83.3	11:28	:28	E	2:15	M&S	Breech extraction.
77	147.8	105.8	41:31	2:31	E	2:05	M&S	Low mid-forceps, mod. Scanzoni.
39	148.3	121.9	16:16	2:16	E	1:55	M&S	Low forceps.
59	153.0	126.1	14:40	1:25	E	2:25	M&S, GOE	Low mid-forceps.
60	204.8	151.4	9:55	1:40	E	2:00	M&S	Low mid-forceps, episiotomy
Mean	123.7	104.1	15:20	1:39				

The mean of 50 maternal blood sugars is 124.6; for 47 infants, 103.6.

The Pearson rank-order coefficient of correlation between maternal and infant blood sugars for 10 multiparæ is +0.66; for 37 primiparæ, +0.59. The coefficient of correlation between duration of labor and maternal blood sugar for 10 multiparæ is +0.32; for 39 primiparæ, −0.17. The coefficient of correlation between duration of ether inhalation and maternal blood sugar for 41 cases which received only ether is +0.15.

* GOE anesthesia consists of a mixture of nitrous oxide, oxygen, and ether.

† GO anesthesia consists of a mixture of nitrous oxide and oxygen.

‡ E, ether.

of the multiparæ and 62.5% of the primiparæ fall between 110 and 140 mg. per 100 cc., figures approximately 50 mg. higher than the normal range for this method.

A further method of ascertaining the effect of anesthesia upon the rise in blood sugar is to exclude the factor of labor while holding the factor of anesthesia constant. In Table 4 are given data on a series of 5 Cæsarean sections, all performed on multiparæ. The anesthetic in all cases was nitrous oxide and ether, and the time given is that from the start of anesthesia to delivery, when determinations were made.

TABLE 4.—DATA ON CÆSAREAN SECTIONS.

Case No.	Fasting blood sugar, mg./100 cc.	Delivery blood sugar, mg./100 cc.		Duration of anesthetic, min.	Pre-operative medication.
		Maternal.	Infant.		
42	91.5	110.2	71.1	25	Pentabarb., gr. iij.
33	102.2	116.3	95.8	15	Sodium amytal, gr. iij.
2	..	117.0	104.6	15	Sodium amytal, gr. iij.
72	87.3	117.7	89.8	25	Sodium amytal, gr. vj.
89	96.5	121.7	84.2	23	Sodium amytal, gr. iij.
Mean	..	116.6	89.1	21	

In this series, the duration of anesthetic is less varied than in any 5 consecutive routine cases, and the range of the maternal blood sugar is distinctly more compact, all falling within the 12 mg. range from 110 to 122 per 100 cc. The mean maternal blood sugar is 116.6, significantly lower than 124.6, the mean of the 50 routine cases. Likewise the mean of 89.1 for these 5 infants is significantly lower than 103.6, the mean for 47 infants in the routine series.

To determine the effect of labor alone 2 patients were delivered by local perineal block with novocaine, which is known to have no effect on the blood sugar. Both of these patients were primiparæ on the morphine and scopolamine medication as previously outlined, and delivery was effected in both cases by low forceps, with episiotomy (Table 5).

TABLE 5.—DATA ON PERINEAL BLOCK CASES.

Case No.	Delivery blood sugar, mg./100 cc.		Labor, hours.		Medication.	Notes.
	Maternal.	Infant.	Total.	Second stage.		
62	106.1	66.0	21:35	:35	M&S	Novocaine, 50 cc.
67	114.4	85.5	9:05	:55	M&S, amyt.	Novocaine, 60 cc.
Mean	110.3	75.7				

The mean maternal blood sugar is 110.3, and that of the infants, 75.7, when labor alone is permitted to operate. Both of these are significantly lower than the means for routine deliveries.

From Tables 4 and 5 it seems that elimination of either labor

or anesthesia produces a rise in blood sugar less than that obtained when both of these factors enter the picture.

A Cæsarean section under local anesthesia eliminated both labor and inhalation anesthesia. This patient, a 23-year-old primipara, had rheumatic heart disease. Pre-operative medication consisted of sodium amytal, grains, vj; novocaine, 1%, was used to effect anesthesia. The maternal blood sugar at delivery was 68.8 mg. per 100 cc., and that of the infant, 65.2. This is lower than any maternal blood sugar found in Tables 3, 4 and 5; thus when anesthesia and labor are both eliminated it would seem that the blood sugar remains within normal range.

From the application of the Pearson rank-order correlation formula to the data presented in Table 3, and from the foregoing data, we feel justified in concluding that labor and anesthesia, alone or combined, cause a rise in the level of the blood sugar at delivery, but it appears that the duration of these factors is not of primary importance in determining the final level attained. Obviously such factors as the rate of excretion of insulin and adrenalin, the severity of labor, available glycogen stores, and the effect of previous diet[5] cannot be overlooked in an analysis of the subject. The interchanging influence of these constituents cannot be evaluated by any research methods now at our disposal. In addition, it has been shown that the blood sugar of the infant is dependent upon that of its mother; factors raising the maternal blood sugar cause a corresponding, but generally smaller, rise in the infant.

TABLE 6.—SUMMARY OF DATA ON BLOOD SUGAR AT DELIVERY.

Type of delivery.	Number of cases.	Mean blood sugar, mg./100 cc.	
		Maternal.	Infant.
Routine forceps delivery . . .	50 maternal 47 infant	124.6	103.6
Cæsarean section, GOE, no labor	5 maternal 5 infant	116.6	89.1
Forceps delivery, local, labor . .	2 maternal 2 infant	110.3	75.7
Morriss, normal, spontaneous, chloroform	28 maternal 24 infant	132.0	115.0
Holman and Mathieu,[1] normal, no other data given	100 maternal 100 infant	100.5	95.4
Cæsarean section, local, no labor	1 maternal 1 infant	68.8	65.2

Table 6 summarizes the blood-sugar values for our cases of various types, and those obtained by Morriss, and Holman and Mathieu. Allowing for the difference in values obtained by the tests used, it is seen that we have verified the work of Morriss. This is interesting in view of the great difference in obstetrical technique, and implies that the blood-sugar values at delivery are not greatly altered by the changes in management of obstetrics.

In 28 of our cases, the blood sugar during the first stage was

compared with that at delivery. These data are tabulated in Table 7, which shows the dilatation by rectal examination at the time of the determination, the delivery blood sugar, and the hours prior to delivery. In every case the blood-sugar value at delivery was higher than during the first stage. There is a tendency for patients showing a high degree of cervical dilatation to present blood-sugar values above normal (50% of patients showing blood

TABLE 7.—COMPARISON OF BLOOD SUGAR DURING THE FIRST STAGE AND AT DELIVERY.

Case No.	Dilatation at time of determination.	Blood sugar, mg./100 cc.		Hours before delivery.
		During first stage.	At delivery.	
73	1 ×	66.2	98.4	3:30
83	4 ×	76.3	102.5	3:00
90	2 ×	78.9	110.0	3:45
46	4 ×	80.5	104.5	3:20
21	1 ×	80.6	135.5	3:45
38	2–3 ×	82.5	103.0	3:50
69	2 ×	84.0	121.8	6:45
61	4 ×	84.3	117.7	3:05
64	Rim	85.4	110.2	6:15
56	None	86.3	116.8	3:00
70	4 ×	86.9	124.8	3:55
74	2 ×	87.1	130.4	7:05
58	2 ×	89.8	114.8	4:07
24	2–3 ×*	90.8	106.6	5:50
87	3 ×	90.9	100.2	3:25
47	3 ×	93.1	124.2	4:00
26	4 ×	96.0	107.4	1:40
63	Rim	100.4	107.5	1:00
13	Rim	100.4	122.5	1:10
80	1 ×	103.0†	133.9	9:45
35	Finger tip	103.7	139.5	4:45
59	Rim	105.8	153.0	2:50
37	1½ ×	106.1	108.8	8:07
29	2 ×	108.2	187.4	7:00
86	4 ×	109.4	136.9	2:45
65	1½ ×	112.6	128.0	7:30
30	Rim	113.2	137.3	2:50
68	2 ×	115.0	127.5	6:10
Mean		93.4	121.8	

* Estimated.

† Taken short time after light meal; not yet returned to fasting; not included in percentages.

sugars over 100 are 4 times or more dilated) while with little or no dilatation patients tend to present values in the normal range. The mean for these 28 cases in the first stage is 93.4, a figure doubtless somewhat raised due to the number of determinations made late in the first stage. The mean blood sugar at delivery is 121.8. We believe the character of the rise in blood sugar during labor needs further study, and therefore reserve for a later date more detailed reports on this phase of the subject.

Comparison of the blood sugar during the first stage with that of the puerperium has had relatively little investigation. Rowley[6]

found a mean of 110 mg. per 100 cc. in 53 cases of pregnancy at term, and a mean of 140 mg. per 100 cc. in 22 cases 2 days postpartum, using the Lewis-Benedict method; apparently these determinations are on different individuals. Morriss, in investigating this phase, likewise did not compare the same individuals during labor and the puerperium, but concluded from determinations on 10 individuals made at varying times postpartum that the blood sugar was slightly higher than normal.

TABLE 8.—COMPARISON OF BLOOD SUGAR VALUES DURING THE FIRST STAGE WITH THAT OF SECOND OR THIRD DAY POSTPARTUM.

Case No.	Dilatation of cervix at time of first stage.	Blood sugar, mg. per 100 cc.		
		During first stage.	2 or 3 days postpartum.	At delivery.
73	1 ×	66.2	97.5	98.4
77	2 ×	74.8	92.8	147.8
83	4 ×	76.3	86.1	102.5
90	2 ×	78.9	97.2	110.0
46	4 ×	80.5	76.2	104.5
38	3 ×	82.5	96.8	103.0
69	2 ×	84.0	84.0	121.8
56	0	86.3	86.4	116.8
70	4 ×	86.9	82.1	124.8
74	2 ×	87.1	78.2	130.4
58	2 ×	89.8	94.7	114.8
87	3 ×	90.9	114.9	100.2
47	3 ×	93.1	84.9	124.2
75	2 ×	97.2	91.2	112.6
63	Rim	100.4	104.8	107.5
80	1 ×	103.0*	82.1	133.9
35	1½ ×	103.7	92.7	139.5
37	1½ ×	106.1	86.6	108.8
86	4 ×	109.4	99.0	136.9
91	4 ×	110.0	63.8	130.5
65	1½ ×	112.6	113.9	128.0
30	Rim	113.2	97.7	137.3
68	2 ×	115.0	73.0	127.5
Mean		93.4	90.3	120.1

* Taken short time after meal; not yet returned to fasting.

Table 8 presents blood-sugar values on 23 patients during the first stage and puerperium. Postpartum blood sugars, either fasting or 4 hours following a light breakfast, were obtained on the second or third day following delivery.

The mean first stage blood sugar in this series was 93.4; the mean postpartum blood sugar, 90.3; the mean blood sugar at delivery, 120.1. The blood sugar at delivery, therefore, represents a temporary rise. It might seem from the mean values that the postpartum blood sugar does not differ widely from that of the first stage of labor. However, inspection of the table shows that, during the first stage, 47.8% of these women had blood sugars above the range for normal adults, and consequently it may be assumed that the level has been influenced by labor. With 2 exceptions, the 11 women having blood sugars higher than the normal range showed a fall in the puerperium. Of the 12 women showing first-stage blood sugars lower than 90 mg.

per 100 cc., 6 show higher values during the puerperium, 5 show approximately the same values and 1 is lower. We conclude that the blood sugar 2 or 3 days postpartum is slightly higher than normal for the individual.

Having followed the mothers into the puerperium, it seemed logical to follow the newborns during the first few days of life. Table 9 gives results of determinations on 37 individual babies at delivery and for 3 consecutive days. The data shows that there is a definite drop in blood sugar during the first 24 hours of life, since the mean delivery blood sugar on these 37 infants was 103.8, and the mean for the first day of life, 67.4. The mean for the second day is 70, practically the same as that of the first day. On the third day there is a slight rise in mean to 75.5. Fifty-nine and a half per cent of the babies show a rise on the second or third day over the first.

TABLE 9.—BLOOD-SUGAR DETERMINATIONS ON NEWBORNS FOR 3 SUCCESSIVE DAYS

Case No.	Blood sugar at delivery.	Blood sugar first day.	Blood sugar second day.	Blood sugar third day.
26	86.4	46.6	59.6	60.8
68	106.1	53.8	60.8	69.2
56	78.2	55.1	70.0	61.8
43	..	55.7	52.4	83.6
44	72.3	56.0	73.5	78.5
46	74.1	56.2	79.5	75.8
5	77.2	58.8	75.2	83.0
7	135.7	60.3	64.5	82.3
41	86.4	61.4	65.7	81.4
49	84.7	61.8	67.0	79.1
58	97.4	62.5	71.6	75.2
69	99.5	62.6	66.2	68.1
45	84.0	63.5	62.4	69.9
12	99.0	64.2	68.9	90.9
54	88.6	64.4	70.0	76.2
55	103.8	65.0	63.9	64.2
6	78.0	65.1	71.5	81.4
47	117.0	66.2	69.7	83.3
36	115.4	68.5	66.2	72.0
38	95.7	68.8	65.0	63.4
22	87.7	69.2	65.0	78.6
65	116.1	70.0	63.0	70.0
37	95.3	70.1	63.9	66.6
70	102.7	70.4	82.3	67.6
28	114.7	71.1	79.1	78.5
60	151.8	72.3	70.6	78.0
66	105.0	73.1	74.5	72.7
64	93.8	74.3	57.4	74.1
34	122.2	74.9	77.1	73.3
59	126.1	75.3	70.2	72.9
39	121.9	75.8	78.5	93.6
9	105.0	76.1	74.8	73.3
8	122.9	76.1	68.3	65.3
11	136.3	78.5	63.2	79.4
30	104.8	80.5	83.8	83.0
51	86.6	82.4	71.3	84.4
10	161.6	87.0	102.9	81.4
Mean	103.8	67.4	70.0	75.5

Blood-sugar collections were made 3½ to 4 hours after feedings.

Using the 37 cases above and adding further cases not necessarily consecutive but falling on the first, second or third day we have established the normal values for newborns with the Folin-Malmros technique (Table 10).

TABLE 10.—FREQUENCY DISTRIBUTION OF BLOOD-SUGAR VALUES IN NEWBORN.

Blood-sugar value, mg./100 cc	Number at each age.				
	At birth.	3 to 6 hours.	First day.	Second day.	Third day.
45– 54.9	0	0	2	1	0
55– 64.9	0	4	16	10	5
65– 74.9	1	4	17	21	14
75– 84.9	8	2	7	8	20
85– 94.9	7	0	2	0	2
95–104.9	9	0	1	1	0
105–114.9	9	0	0	0	1
115–124.9	8	0	0	0	0
125–134.9	1	0	0	0	0
135–144.9	2	0	0	0	0
145–154.9	2	0	0	0	0
Total cases	47	10	45	41	42
Mean (mg./100 cc.)	103.6	66.8	68.3	70.3	76.1
Normal range	..	55–75	55–75	55–75	65–85
Highest value of series	..	79.1	102.2	102.9	110.3
Lowest value of series	..	56.9	46.6	52.4	57.1

Blood-sugar collections were made 3½ to 4 hours after feedings.

Table 10 shows a drop from the high values at birth to a mean value of 66.8 during the first 3 to 6 hours. The mean values 3 to 6 hours after birth, and on the first and second day are practically the same; on the third day there is a small but appreciable rise. There is also a rise in the normal range. The lowest blood sugar of the series, 46.6, was not attended by clinical signs of hypoglycemia. We conclude, therefore, that the normal blood-sugar values for newborns by the Folin-Malmros technique is somewhat lower than the normal values for adults, and lies between 55 and 75 mg. per 100 cc. for the first 2 days of life with slightly higher values later.

Conclusions. 1. The Jeghers-Myers modification of the Folin-Malmros micro method for blood sugar is an accurate and practical test for use with adults and newborns; 85% of the duplicates check within 3%.

2. The mean maternal blood sugar at delivery in 50 cases was 124.6, with 60% of the cases falling between 110 and 140 mg. per 100 cc., approximately 40 to 50 mg. above the normal range.

3. The mean blood sugar for 47 infants at birth was 103.6; 94% of these infants presented values lower than those of their respective mothers.

4. Both anesthesia and labor have been shown to contribute to the rise in blood sugar at delivery, but there is no indication that the duration of these factors is the primary cause of the rise.

5. The blood sugar of the infant is dependent upon that of its mother, and factors affecting the blood sugar of the mother affect that of the infant.

6. Taking into account the method used in determining blood-sugar values, we have confirmed the work of Morriss (1917). Inasmuch as the management of labor and delivery is entirely different in the two series, it would seem that the newer obstetrical techniques produce no deleterious effects on the glucose metabolism.

7. In 28 cases, comparison showed the blood sugar higher at delivery than during the first stage by a difference in mean of 28 mg. per 100 cc.

8. The blood sugar of any particular individual 2 or 3 days postpartum is somewhat higher than normal.

9. We have established the normal blood-sugar range for newborns by the modified Folin-Malmros technique. The first 2 days of life the blood sugar is 55 to 75 mg. per 100 cc., with slightly higher values on the third day.

10. Within 3 to 6 hours after birth the blood sugar drops to the normal infant range.

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THE BLOOD AND URINE OF DOGS FOLLOWING PARALDEHYDE.

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Judging from the meager amount of information which appears in the literature,[2,11] little is known of the changes which occur in certain constituents of the blood and urine following the administration of paraldehyde. Since this drug has assumed renewed importance in recent years, particularly in the field of obstetrics,[4,6,7,9,10] it was felt that additional studies of this nature were necessary.

Our first investigations were concerned with the effects produced

by paraldehyde on the amounts of fermentable and non-fermentable reducing substances of the blood, by means of which for the first time, so far as is known, the changes which occurred were definitely identified as being due to glucose fluctuations. Later these investigations were extended to include the urine. When it became apparent that the blood showed an increased viscosity during the anesthetic stages, and clotted more readily, changes in the red cell volume were studied by hematocrit determinations, since blood volume changes might affect the blood sugar content.[12] The carbon dioxide combining capacity of the blood plasma was also studied during the course of several experiments, to determine the extent of changes in the acid : base balance, which have been observed to occur with other anesthetics.

Experimental Procedure. The animals used were healthy adult dogs, weighing 7 to 17 kgs. They were fed *ad libitum* on a brand of prepared dog food, occasionally supplemented with raw meat and cod-liver oil. All food was removed 18 hours before experimental procedures were begun. The experiments were usually continued for 6 hours, samples of venous blood and catheterized urine being obtained at hourly intervals. Clotting was prevented by the addition of 2.5 mg. of powdered potassium oxalate per cc. of blood, the minimum amount found necessary for this purpose.

The normal average daily variation of the blood sugar level was determined three times at weekly intervals on each of 6 dogs from samples removed during the 6-hour period at 9.00 A.M., 12.00 M., and 3.00 P.M. In the case of the experimental animals, after control samples of blood and urine had been obtained, an anesthetic dose of paraldehyde, usually 1.8 cc. per kg.,[5] was administered either by stomach tube or slowly by rectum. When given by the latter route the rectum was first emptied by irrigation, and 1.5 cc. of benzyl alcohol was mixed with the paraldehyde to minimize local irritation.[6] The animal was held in a prone position until there was no danger of loss of the drug from the rectum; in no instance did vomiting occur following gastric or rectal administration.

The majority of the experiments were conducted at room temperature, with the animals covered sufficiently to avoid excessive loss of body heat. In those tests where the effects of moderate extremes of temperature were also investigated, heat was supplied by electric heating pads, and cold by exposure to cold air from an open window, such measures being initiated 1 hour after paraldehyde had been given, and the animals were quiet.

The total amount of reducing substances in the blood was determined by the method of Benedict;[3] the non-fermentable reducing substance was estimated by the same procedure after yeast fermentation. The methods used for determining these substances in the urine were identical except that dilution of the urine from 60 to 80 times was necessary, depending on the concentration of reducing substances and pigment present in the particular sample.

The red cell volume (hematocrit) determinations were made by centrifuging the freshly oxalated blood in glass tubes sealed at one end, until no further settling occurred. For this purpose pieces of glass tubing 7 cm. in length with an inside diameter of $1\frac{1}{2}$ or 2 mm. were used, a small drop of mercury being placed in the sealed end to fill the tapering bore and give a uniform meniscus. Readings were obtained with a millimeter rule and magnifying glass. Although the use of oxalate causes a slight shrinkage of the red cells[8] resulting in a slightly smaller volume reading, this can be disregarded since it is constant in each test.

While it is obvious that there is no exact method of measuring the degree of hypnosis or anesthesia, the following symptomatic classification appeared to give a fair evaluation, and served satisfactorily as a basis for construction of the "anesthesia curves" shown in the figures. Induction stage: animal awake, either standing or reclining, muscular incoördination, quiet or struggling. Light anesthesia: animal asleep, but awakens when needle penetrates the skin. Complete anesthesia: animal unconscious, breathing slow, deep and regular, reflexes active, does not react to needle puncturing the skin.

Results. *Effect of Paraldehyde on the Blood Sugar Level.* The maximum fluctuation of the blood sugar levels of 6 fasting control dogs over the 6-hour period averaged about 7 mg. %, and never exceeded 10 mg. % in any one animal in the control tests on 18 separate days.

Since 1.5 cc. of benzyl alcohol was always mixed with paraldehyde when the latter was given by rectum, it was first necessary to determine whether or not the former had any effect on the blood sugar level. Hourly fluctuations which occurred in such experiments on 2 dogs were so slight and inconstant as to be of no significance, so that benzyl alcohol need not be considered as a factor in the production of these changes.

TABLE 1.—THE BLOOD SUGAR LEVEL FOR 6 HOURS AFTER PARALDEHYDE.

Dog No.	No. of tests.	Paraldehyde.		Blood sugar (mg. %).						
		Amount (cc. per kg.).	Route—"r": rectum, "s": stomach.	Control.	Hours after administration.					
					1.	2.	3.	4.	5.	6.
37 . . .	5	1.7	5 r	76	92	83	81	82	76	85
38 . . .	4	2.7	3 r, 1 s	63	73	73	77	79	78	67
39 . . .	3	1.5	3 r	67	77	72	70	73	72	65
55 . . .	7	1.8	4 r, 3 s	72	93	79	78	70	70	73
63 . . .	2	1.8	2 s	77	120	106	97	93	95	93
65 . . .	1	1.8	1 s	66	74	83	89	94	96	80
66 . . .	7	1.8	3 s, 4 r	79	88	78	74	75	77	79
72 . . .	1	1.8	1 s	75	111	136	167	154	139	128
75 . . .	6	1.8	2 s, 4 r	73	91	83	79	96	96	88
88 . . .	3	1.8	3 r	72	77	78	81	84	82	79
98 . . .	5	1.8	5 r	78	105	111	108	109	108	102
99 . . .	4	1.8	4 r	71	76	77	81	87	78	78
Totals . .	48	. . .	13 s, 35 r	. . .	. . .	. . .	. . .	. . .	. . .	. . .
Average .	. . .	1.8	. . . . .	73	89	84	84	85	84	82

The administration of 1.8 cc. of paraldehyde per kilogram of body weight by stomach or by rectum produced a small average rise of the blood sugar level in 48 experiments on 12 animals (Table 1). The rise averaged 16 mg. % at the end of the first hour, and maintained an average increase of 11 mg. % above normal during the remaining 5 hours of observation. Some individual variations from the average

blood sugar level occurred, such as secondary rises, falls below normal after a preliminary rise, and slow progressive elevations. These variations which perhaps depend to some extent upon individual reaction to the drug, are probably due mainly to differences in the rate of its absorption, since the blood sugar level in some instances varied considerably in character in the same animal on different days. The maximum rise of the blood sugar level generally occurs during the partial development of anesthesia in the first hour; a slight fall occurs during the second hour as the anesthetic effect reaches its maximum, and the lower level which is maintained during the remaining 4 hours parallels the anesthetic effect.

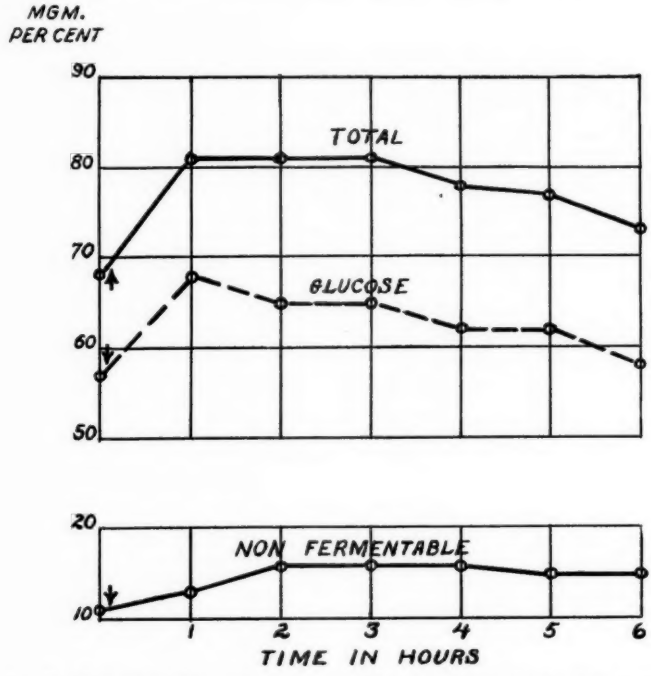

FIG. 1.—Total, fermentable and non-fermentable substances in the blood of dogs following paraldehyde (1.8 to 4 cc. per kg.). (↑ = paraldehyde administered.)

Partition of Reducing Substances in the Blood Under Paraldehyde. Early in our investigations it was found that the Benedict reagent is reduced by paraldehyde, and since it was possible that sufficient amounts of the latter might be present in the blood to partially account for the reduction obtained, simultaneous determinations of the total and non-fermentable reducing substances in the blood were made in 6 experiments following paraldehyde, using the yeast fermentation technique described above. Even though larger amounts of paraldehyde were administered than usual, it was found

that the fluctuations in the amount of non-fermentable reducing substance were insufficient to account for the changes in the reducing power of the blood (Fig. 1). It can therefore be concluded that these changes were due to variations in the amount of glucose, and not to other non-fermentable reducing substances, or to paraldehyde.

The Relation of External Temperature to the Blood Sugar Level Under Paraldehyde. While most of our experiments were performed at room temperature, the results of the investigations reported in Table 2 on 6 dogs are from tests designed to determine what influence, if any, was exerted by external temperature on the blood sugar level after paraldehyde. In experiments at room temperature it had been observed frequently that after receiving this drug some of the animals exhibited trembling movements even when well covered; it was therefore felt necessary to determine whether these were shivering movements due to thermal conditions, or muscular movements brought about by some other action of the drug, and what was their effect on the blood sugar level. Accordingly, two experiments were performed on each of 6 animals; in one test the animal was kept warm with electric pads and blankets, the temperature being held just low enough so that the animal would not pant;[1] in the other test it was uncovered and exposed to the

TABLE 2.—INFLUENCE OF TEMPERATURE ON THE BLOOD SUGAR LEVEL FOLLOWING PARALDEHYDE (1.8 CC. PER KG., RECTALLY).

Dog No.	No. of tests.	Blood sugar (mg. %).							External temperature.*
		Control.	Hours after administration.						
			1.	2.	3.	4.	5.	6.	
55 . .	1	65	78	59	64	48	53	70	High
	1	69	87	72	70	65	50	49	Low
66 . .	1	76	98	78	78	83	77	81	High
	1	76	84	74	70	74	76	79	Low
75 . .	1	75	106	93	78	86	84	82	High
	1	73	87	78	74	96	86	88	Low
88 . .	1	64	67	71	76	(animal awake)			High
	1	72	78	81	85	84	82	89	Low
98 . .	1	71	159	149	128	111	106	95	High
	1	80	90	146	154	137	127	115	Low
99 . .	1	68	79	76	77	74	60	70	High
	1	72	76	82	77	85	82	79	Low
Averages of 6 high		70	98	88	83	80	76	80	
Averages of 6 low		74	84	89	88	91	84	82	

* Second to sixth hour, inclusive.

winter cold in front of an open window at temperatures ranging −1 to +13°C. and averaging 6° C., so that it shivered violently throughout the experiment. These procedures were employed just after the blood (and urine) samples had been taken at the end of the first hour following the administration of paraldehyde, so that the results in Table 2 are significant in respect to temperature influence beginning with the second hour only.

While the average results indicate that the blood sugar level under paraldehyde was slightly higher when the animal was exposed to cold than when kept warm, this was true in only half of the experiments. Trembling was also observed in some of the animals which were kept warm. It would therefore appear that the room temperatures at which most of the tests were performed were entirely satisfactory, the blood sugar changes after paraldehyde not being attributable to variations in room temperature. It also seems apparent that the trembling movements are primarily induced by some other action of paraldehyde than lowering of temperature, since they occurred at times when the animals were warm, and often were absent when the tests were run at room temperature. However, these trembling movements were either intensified or changed into shivering movements when the animals were exposed to low temperatures.

Abnormal Elevation of the Blood Sugar Level in a Poorly Nourished Dog Under Paraldehyde. The results obtained on Dog 98 are

TABLE 3.—ABNORMAL ELEVATION OF THE BLOOD SUGAR LEVEL OF A POORLY NOURISHED DOG (NO. 98) FOLLOWING PARALDEHYDE (1.8 CC. PER KG. RECTALLY).

Date.	Weight (kg.).	Blood sugar (mg. %).							External temperature.*	Condition.
		Control.	Hours after administration.							
			1.	2.	3.	4.	5.	6.		
12/22/36	10.7	75	76	75	85	108	121	125	Room	Thin
1/15/37	11.0	71	159	149	128	111	106	95	High	Thin
2/4/37	11.0	80	90	146	154†	137†	127	105	Low	Thin
3/29/37	14.3	82	94	91	81	78	82	86	Low	Robust
5/7/37	15.0	82	105	96	94	111	106	99	Room	Robust

* Second to sixth hour, inclusive.
† Glycosuria occurred.

interesting because of the unusually high elevations of the blood sugar level which occurred under paraldehyde when this animal was in a poorly nourished condition (Table 3). These marked increases occurred in the first 3 experiments when the animal was very thin, but otherwise apparently normal, similar results being obtained even under opposing extremes of external temperature. After the animal had gained weight the small changes in the blood sugar level

under paraldehyde approximated those obtained in the other normal animals. No abnormal conditions were found at autopsy when the animal was sacrificed after the last experiment. Sugar occurred in the urine in the third experiment only, at blood sugar levels slightly lower than in the preceding experiment where this event did not occur.

The Amount of Reducing Substances in the Urine Under Paraldehyde. The amount of non-fermentable reducing substances in

TABLE 4.—THE AMOUNT OF REDUCING SUBSTANCE IN THE URINE FOLLOWING PARALDEHYDE (1.8 CC. PER KG., RECTALLY).

No. of animals.	No. of tests.	Reducing substance (in mg.).							Type.
		Control.	Hours after administration.						
			1.	2.	3.	4.	5.	6.	
6 . . .	13	25	44	58	68	127	135	101	Non-fermentable
1 . . .	1	..	..	..	59*	249*	...	...	Fermentable (glucose)

* Glucose present in urine in one experiment on 1 animal (Dog 98).

the urine following paraldehyde administration showed a progressive increase during the 6-hour period in the 13 tests performed on 6 animals (Table 4). Fermentable reducing substance (glucose) was present in only 1 experiment. Since the Benedict reagent is reducible by paraldehyde, it appears probable that the latter forms part of the non-fermentable reducing substances, and that its excretion may largely account for the increased reducing power of the urine.

The concentration as well as the amount of non-fermentable reducing substances in the urine showed a progressive increase, since the rate of urine output remained about the same over the 6-hour period.

Effect of Paraldehyde on the Red Cell Volume (Hematocrit). Hematocrit determinations in 24 experiments on 6 dogs (Fig. 2) revealed a 3% decrease in the red cell volume in the first hour following paraldehyde. The red cell volume then increased progressively until it was 3% above normal at the end of the fourth hour, and declined toward normal in the last 2 hours as anesthesia lessened. The red cell volume apparently is unrelated to the blood sugar curve.

Effect of Paraldehyde on the Carbon Dioxide Combining Capacity of the Blood Plasma. The carbon dioxide combining capacity of the blood plasma was determined in 5 experiments on different animals following paraldehyde administration (Fig. 3). The results obtained on Dog 99 are plotted separately, since this animal showed only slight hypnotic effects, mainly during the first and second

hours, due to loss of some of the drug from the rectum, and had almost entirely recovered by the end of the experiment.

The carbon dioxide combining capacity decreased progressively during the first 5 hours and began to recover during the last hour, in the 4 experiments where good anesthesia occurred. In Dog 99 the decrease in this property was much less marked, with an earlier recovery.

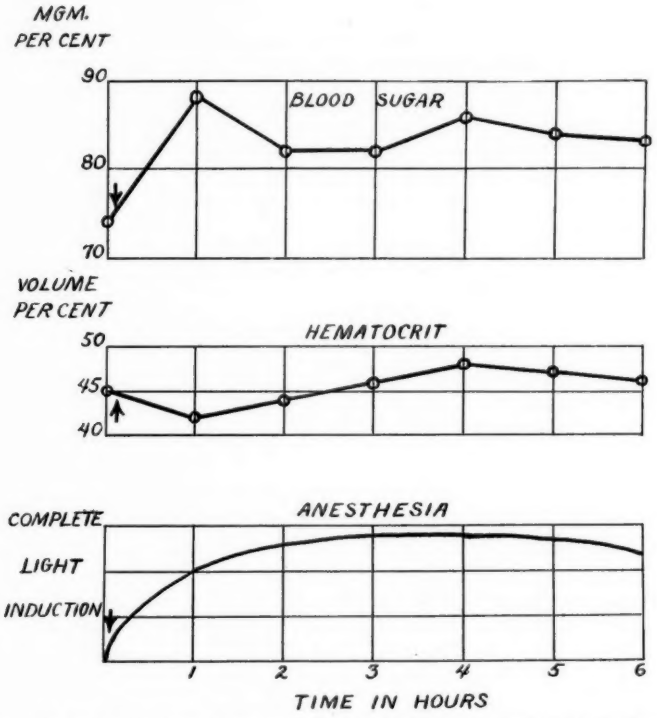

FIG. 2.—The red cell volume (hematocrit) and the blood sugar level of dogs following paraldehyde (1.8 cc. per kg.). (↑ = paraldehyde administered.)

The hematocrit curves included in the chart indicate that the usual changes in red cell volume occurred only during good anesthesia. Changes in both the red cell volume and carbon dioxide combining capacity were smaller and less constant in the experiment on Dog 99 in agreement with the brief and mild hypnotic effect.

Discussion. While numerous investigators have shown that anesthesia produced by ether, chloroform, nitrous oxide and ethylene is accompanied by a rise in the blood sugar level and a reduction in the plasma bicarbonate, the results reported on similar studies on the large group of barbiturates are often quite contradictory, and a search of the literature has revealed only two such references on

paraldehyde. Sollmann[11] states that Powell, in 1914, found hypnotic doses of paraldehyde lowered the blood sugar level in dogs, while anesthetic doses produced considerable hyperglycemia and glycosuria. Beauchemin, Springer and Elliott[2] recently reported that 5 to 19 cc. of paraldehyde, rapidly injected intravenously in human insane patients, produced good anesthesia lasting 1½ to 21 minutes, accompanied by hyperglycemia (maximum of 30 mg. %) which began to fall after 1 hour, and disappeared by the end of 24 hours.

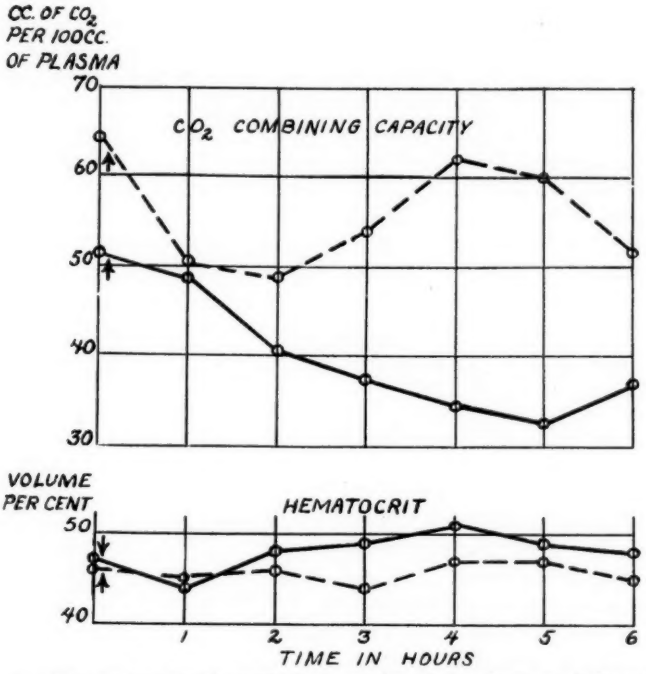

FIG. 3.—The carbon dioxide combining capacity of the plasma and the red cell volume of the blood of dogs following paraldehyde (1.8 cc. per kg.). (Broken line, Dog 99, mild hypnosis only. Solid line, average of Dogs 55, 66, 75, 98, good anesthesia. ↑ = paraldehyde administered.)

The relatively small elevations of the blood sugar level which occurred in our experiments on dogs with paraldehyde, as compared to those obtained by numerous investigators with various other anesthetics, can probably be accounted for on the basis of milder anesthetic activity and slower action of this drug. The results of our investigations do not confirm those ascribed to Powell above, since our anesthetic doses produced only a mild hyperglycemia, and no glycosuria in healthy normal animals. In the one experiment where only light hypnosis was obtained due to loss of the drug from the rectum (Dog 99), the elevation of the blood sugar level was similar in degree to that which followed the anesthetic dose.

In our experiments there was relatively little excitement or struggling during the induction of anesthesia; during the anesthetic stage there were only moderate changes in the respiration, neither hyperpnea nor dyspnea occurring. The possible effects of extremes of external temperature, through stimulation of muscular tone and activity (shivering) and stimulation of respiratory activity (panting) have been shown to be unimportant in regard to changes in the blood sugar level under paraldehyde in these experiments.

There was a gradual decrease in the carbon dioxide combining capacity of the blood plasma from the first to the fifth hour, with a slight return to normal in the fifth to sixth hour. These findings indicate that a mild to severe acidosis was produced according to the classification of acidosis by Van Slyke.[13]

The primary decrease in red cell volume in the induction of paraldehyde anesthesia, followed by an increase in the anesthetic stage, is the reverse effect to that which occurred in the blood sugar level. It therefore appears that the changes in blood sugar level observed were not due to variations in the red cell volume.

Summary. 1. The gastro-enteral administration of anesthetic doses of paraldehyde (1.8 cc. per kg.) to healthy fasting dogs produced a small but definite average elevation of the blood sugar level. In general, this elevation was greatest during the first hour, averaging 16 mg. %; during the remaining 5 hours a level which averaged 11 mg. % was maintained. Occasionally the level approached or fell below normal during the later hours; in some cases a secondary rise occurred, while in others the rise was delayed and continued throughout the experiment.

2. By means of yeast fermentation technique it was definitely ascertained that the elevations in the reducing substance of the blood observed under paraldehyde were entirely attributable to increases in the amount of glucose.

3. A small amount of benzyl alcohol (1.5 cc.), administered rectally with paraldehyde to minimize the irritating effect of the latter, had no effect on the blood sugar level; nor was the character of the blood sugar level under paraldehyde altered by moderate extremes of cold and warmth in the external temperature.

4. High elevations of the blood sugar level (50, 78 and 74 mg. %) occurred in a thin and poorly nourished animal under paraldehyde anesthesia; however, when the animal had gained weight and was in good condition, the usual small increases in blood sugar were obtained under the same procedure.

5. Glycosuria did not occur in normal dogs during paraldehyde anesthesia, nor were there significant quantitative changes in the rate of urine secretion. There was, however, a marked progressive increase in the amount of non-fermentable reducing substance in the urine, the nature of which was not determined, but which is suspected of being paraldehyde or its decomposition products.

6. The red cell volume (hematocrit) of the blood showed an average decrease of 3% at the end of the first hour following paraldehyde, then rose progressively to a maximum of 3% above normal in the fourth and fifth hours, returning to normal by the end of the sixth hour.

7. The carbon dioxide combining capacity of the blood plasma during paraldehyde anesthesia was lowered to a degree corresponding to moderate to severe acidosis (32.5 cc. per 100 cc. of plasma).

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OBSERVATIONS ON TWO DIFFERENT PRESSOR SUBSTANCES OBTAINED FROM EXTRACTS OF RENAL TISSUE.*

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Since the time of Bright most clinicians have believed that certain disorders of the kidney may lead to an increase in blood pressure. This concept has recently received experimental support from the work of Goldblatt[7] and his colleagues, who have shown that partial obstruction of the renal arteries produces hypertension in dogs, an observation which has been confirmed by Page,[16] Friedman and Prinzmetal,[6] and others.[4,9] Hartwich[11] observed increase in blood pressure following ligation of the ureters. His results are confirmed by Harrison *et al.*,[10] who failed to obtain comparable increase in blood pressure after bilateral nephrectomy, and concluded that the development of hypertension was dependent in some manner on the presence of malfunctioning renal tissue in the body and was not related to failure of the renal excretory mechanism.

* This study was aided by a grant from the Division of Medical Sciences of the Rockefeller Foundation.

The "renal" hypertension occurring under the conditions mentioned above might be the result either of a reflex initiated in the kidneys or of some chemical substance elaborated in these organs. That the former hypothesis cannot be correct is indicated by the experiments of Page,[16] Elaut[4] and of Collins,[2] who showed that denervation of kidneys did not prevent the rise in blood pressure following the application of a Goldblatt clamp to the renal arteries, and Harrison and his coworkers, who found that ligation of the ureters caused a rise in blood pressure regardless of renal denervation. By exclusion, it seems that "renal" hypertension is dependent on the pressor effects of some chemical agent elaborated in the kidney. Attempts to demonstrate such a substance in the blood of hypertensive animals have led to conflicting results. Govaerts and Dicker[8] obtained positive effects while negative findings have been reported by Friedman and Prinzmetal[6] and Page.[16] On the other hand, pressor effects from the kidneys of hypertensive animals have been reported by Hartwich,[11] Harrison, Blalock and Mason,[9] and by Friedman and Prinzmetal.[6] These authors, dealing with renal saline extracts, obtained greater pressor effects from the kidneys of hypertensive than from normal animals. However, since these extracts also had well-marked depressor effects, the findings were not entirely convincing and it was felt that the experiments should be repeated with extracts freed from confusing depressor substances. The observations to be reported in the present paper deal with attempts to purify the pressor principle of renal extracts.

In 1898, Tigerstedt and Bergman[22] reported that the intravenous administration of saline extract of rabbits' kidneys to other rabbits caused a 10 to 50% rise in blood pressure, which came on slowly and had a long duration. The active substance, which they called "renin," was non-dialysable, and heat labile. It was found chiefly in the cortex of the kidney, and was still effective after cervical chordotomy and vagal section. These findings, while not completely confirmed by some authors,[15,18] have been substantiated by Bengel and Strauss[1] and Thauer,[21] and Hartwich and Hessel.[12,13] Bengel and Strauss obtained "renin" from renal press-juice rather than from saline extract. They concluded that it was soluble in water, non-dialysable, precipitable by ammonium sulphate in proper concentration and seemed to be a protein. Hessel and Hartwich, working with autolyzed press-juice, confirmed the results of Bengel and Strauss. However, they found that when autolysis was allowed to go on several weeks, the pressor effect came on more quickly, was greater in degree and shorter in duration. They observed that while organic solvents did not remove the pressor agent from fresh press-juice, such solvents did extract a highly potent pressor principle when used on autolyzed press-juice. From the older autolysates a pressor principle was readily obtained by ultrafiltration while the ultrafiltrate of fresh press-juice was inactive. They explained

their findings by assuming that "renin" was bound in combination with a protein and that this combination was broken up by autolysis. They concluded that the pressor agent obtained from their autolysates was a small molecular basic substance, probably an amine. Recently Hessel and Maier-Hüser[14] claim to have prepared "renin" in a highly purified form and administration of minute doses (0.2 mg.) to dogs caused a pronounced and sustained rise in blood pressure. No information was given concerning the method of purification.

The experiments to be described were carried out for the purpose of learning more concerning the nature of the renal pressor substance in the hope that by this means it might be possible to make assays of the pressor content of hypertensive kidneys uncomplicated by the confusing effects of coëxisting depressor agents.

Methods. With dogs as test subjects, it was found that anesthesia had a pronounced influence on the results. Extracts which were pressor in unanesthetized dogs sometimes produced only a decline in blood pressure when injected into anesthetized animals. (This was later found to be due to increased sensitivity of the anesthetized animals to depressor bodies also in the extracts rather than to a reversal of the effect of the pressor agent.) Hence, all the experiments described were carried out on unanesthetized dogs especially trained for these experiments. Most of the blood-pressure measurements were made by means of a cuff similar to that described by Ferris and Hynes,[5] applied to the hind leg, the passage of the pulse wave being determined by palpation of the dorsal artery of the foot.

When an extract was to be tested, the dog with the blood-pressure cuff in place was allowed to rest on his side on the table for several minutes. Seven measurements of blood pressure were than made, the highest and lowest readings discarded and the average of the other 5 measurements considered as the control value. When any considerable variation in these readings was encountered a second set of control readings was taken, usually by a different observer. After it was apparent that the dog's blood pressure was constant the material to be tested was administered into a superficial vein of one of the legs. Following the injection the blood pressure was measured at intervals of $\frac{1}{2}$ to 1 minute. The rise in blood pressure was considered to be the difference between the average of the 3 highest successive readings after the injection and the average control value.

A few observations were made with a cannula in the femoral artery, using local novocaine anesthesia.

It was found that some unanesthetized dogs, while remaining perfectly quiet, exhibited well-marked spontaneous changes in blood pressure. Furthermore, it was observed that different dogs displayed marked variation in the response of the blood pressure to injections of the same preparation. Because of these variables mentioned it was not possible to obtain constant effects. With each of the various preparations used, occasionally depressor responses, or no response at all, were obtained and test fractions which ordinarily were inactive, or depressor, occasionally were pressor. Such variations were inherent in incompletely purified preparations, especially when one is using unanesthetized animals, and it is not believed that the occasional atypical responses can be considered as seriously impairing the conclusions to be drawn. In reporting the results we have depended on the effects obtained in the great majority of instances from a given preparation.

Fresh pig kidneys were obtained from the slaughter house. Cortex and medulla were quickly separated, the cortex passed through a meat grinder, and ground in a mortar with carborundum and 2 cc. of physiologic saline per gram of cortical tissue. After 10 to 20 minutes' grinding the mixture was centrifuged and the supernatant suspension separated and employed for injection or for various extraction procedures described below. The amount of the final extract injected usually corresponded to 10 or 20 cc. of saline extract, *i. e.*, 5 or 10 gm. of the original kidney cortex.

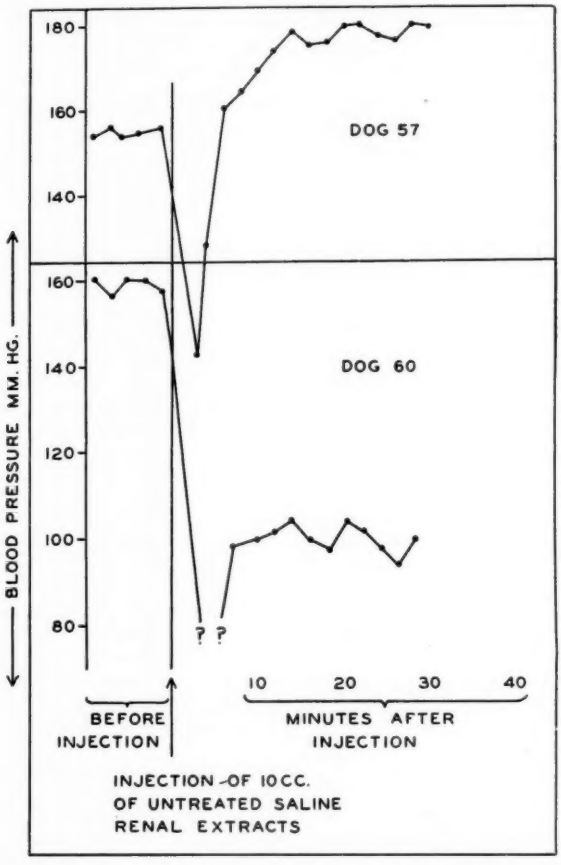


FIG. 1.—The upper part of the figure illustrates a typical result—a temporary fall in blood pressure being followed by a sustained increase. The lower part illustrates an experiment in which only the depressor effect was observed.

Results.—*Saline Extract.* Several score experiments were performed with varying results. In most of the experiments a pronounced and occasionally fatal fall in blood pressure occurred immediately after the injection. Often the blood pressure remained low for several minutes, gradually returning to the normal level. Frequently the preliminary decline in blood pressure was followed

by a prolonged and sustained elevation and in some instances a rise occurred without the preliminary decline. The maximum increase was not usually observed for several minutes and it was usually sustained for 20 minutes or longer before gradually returning toward the control level. Some dogs reacted regularly with a depressor effect, others with a diphasic result and still others showed only the pressor response. More commonly a given dog displayed varying responses. Illustrative experiments are shown in Figure 1.

Fractions Obtained by Precipitation With Ammonium Sulphate. Bengel and Strauss[1] found that the active pressor substance was not

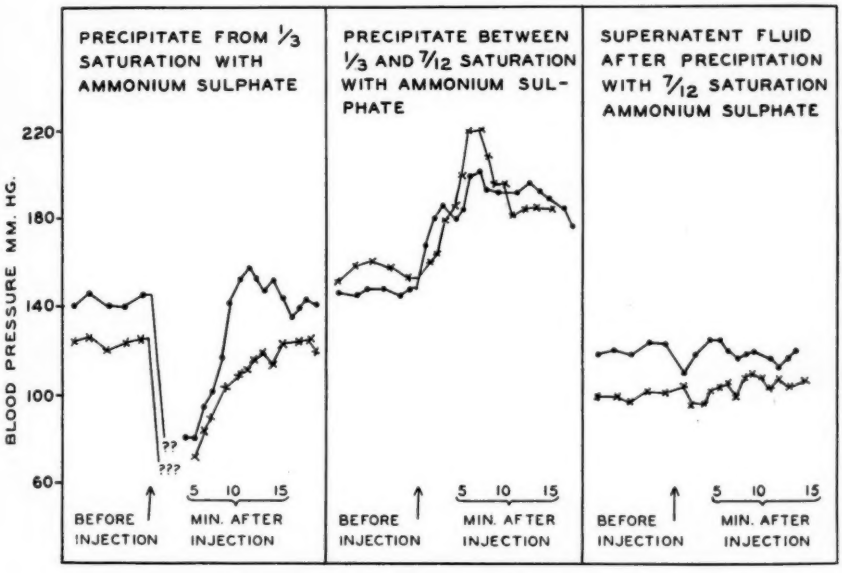


FIG. 2.—The chart shows the effect of various fractions of renal extract after treatment with ammonium sulphate. The precipitate from 1/3 saturation was usually depressor; the fraction precipitated between 1/3 and 7/12 saturation was usually pressor; the fraction remaining in solution after 7/12 saturation was inactive.

precipitated by 1/3 saturation of ammonium sulphate but did come down with 7/12 saturation of this salt. Consequently, preparations were made as follows: Ammonium sulphate was added to 1/3 saturation to the fresh saline extract. After standing overnight the suspension was centrifuged, the supernatant fluid made up to 7/12 saturation and again allowed to stand overnight. The two precipitates—that coming down with 1/3 saturation and that brought down between 1/3 and 7/12 saturation—were then suspended in water and both of them as well as the supernatant fluid from the 7/12 saturation were dialyzed for 12 to 15 hours against flowing

tap water.* The three fractions were then tested for their pressor properties. Although some variation occurred in different experiments the general trend of the results, which is illustrated in Figure 2, was quite clear. The precipitate at 1/3 saturation usually gave only depressor effects, that between 1/3 and 7/12 saturation was ordinarily pressor, and the final supernatant fluid was inactive. These results, in agreement with those obtained by Bengel and Strauss[1] and by Hartwich and Hessel,[12] indicate that the active principle has the properties of a protein.

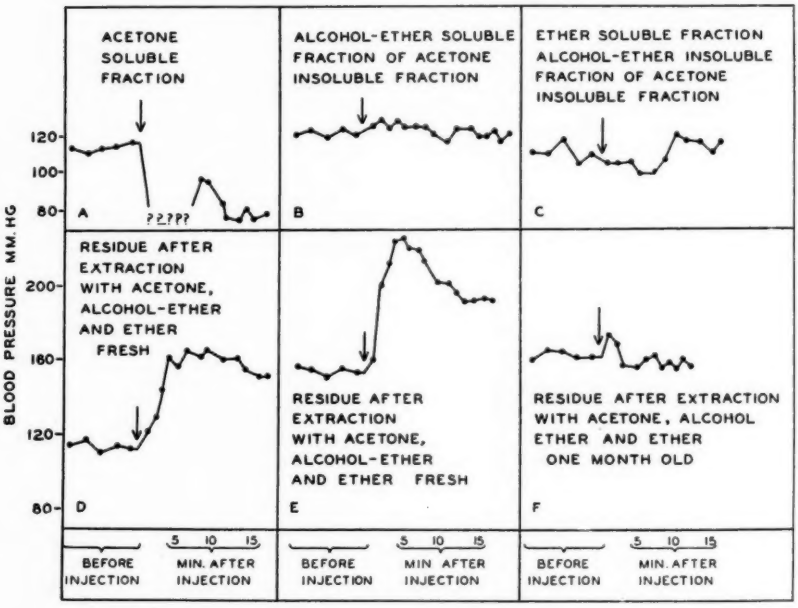


FIG. 3.—Fresh renal extract was successively treated with acetone, alcohol-ether mixture, and ether. The acetone-soluble fraction was usually depressor (*A*). The alcohol-ether and ether-soluble fractions were inactive (*B* and *C*). When the residue left after extraction with these organic solvents was taken up in physiologic saline solution a pressor effect was obtained (*D* and *E*). This pressor agent was unstable (*F*).

Fractions Obtained by Precipitating With Acetone. Five volumes of acetone were added to 1 volume of the fresh saline extract, the mixture was shaken for 5 minutes, allowed to stand for an hour and centrifuged. The precipitate was shaken with alcohol-ether solution (3 parts alcohol to 1 part ether) equal to the volume of the original saline extract. After centrifuging, the precipitate was shaken with ether and again centrifuged. The final precipitate was dried and pulverized in air and immediately shaken with 0.9%

* Non-protein nitrogen determination on these dialyzed preparations showed them to be practically free of ammonium sulphate.

sodium chloride and centrifuged, the supernatant fluid being employed for injection.

Typical results obtained by the injection of these several fractions are shown in Figure 3. The acetone-soluble fraction (after removal of the acetone *in vacuo*) was either inactive or, more commonly, depressor. The alcohol-ether and ether-soluble fractions (after removal of these solvents) were inactive. The saline extract of the final residue after extraction with the above solvents usually gave a

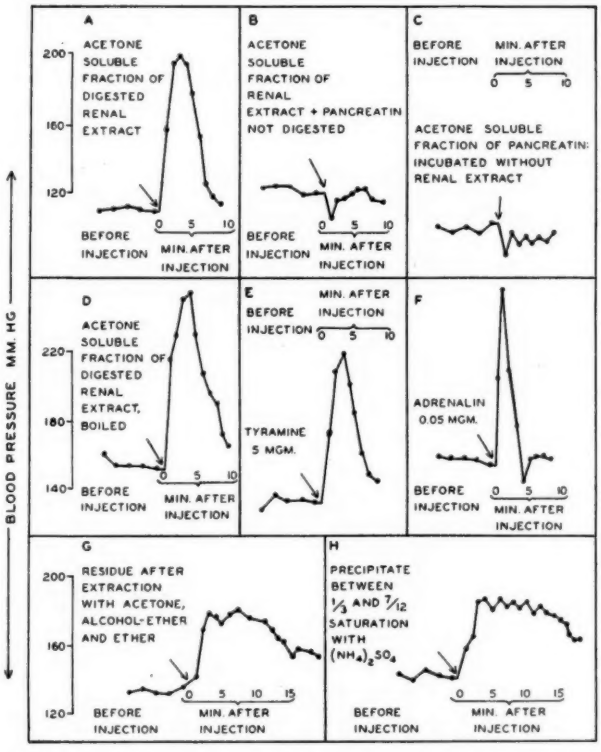


FIG. 4.—After digestion of renal extract with pancreatin, an acetone-soluble, powerful pressor agent was obtained (*A*). Material similarly treated but not digested gave no such effect (*B*). Incubation of pancreatin solution alone yielded negative results (*C*). The active pressor agent was heat-stable (*D*) and its effect was identical with that of tyramine (*E*), being more lasting than that of adrenalin (*F*) and more evanescent than that of the pressor substances obtained by precipitation with acetone (*G*) or with ammonium sulphate (*H*).

slowly developing, sustained and fairly pronounced pressor effect (Fig. 3, *d* and *e*). The blood pressure raising principle obtained by this method was quite unstable (Fig. 3, *f*), and on standing at room temperature rapidly became inactive. It was also noted that when the original saline extract was several days old the final preparation

usually had a pronounced depressor action, or would be totally inactive.

Whether the active principle obtained by this method is identical with that obtained by ammonium sulphate precipitation is uncertain. Their effects on blood pressure were similar; however, the pressor agent obtained by precipitation with acetone was much less stable than that secured by treatment with ammonium sulphate.

Fractions Obtained by Digestion. It was found by Hessel and Hartwich, as mentioned above, that when renal press-juice was allowed to autolyze for several weeks at 37° C., the pressor substance was ultrafiltrable. In an attempt to reproduce these results more quickly we have studied the effects of digested renal saline extract. Freshly prepared extract was adjusted with phosphate buffer and sodium bicarbonate to approximately pH 8. Pancreatin was added and the mixture incubated at 37° C. for 12 to 16 hours. The digest was found to be powerfully depressor; however, when it was extracted with acetone the acetone-soluble fraction proved, after removal of the remaining depressor agents by extraction with petroleum-ether and removal of the solvents, to contain a pressor substance. This pressor effect was not obtained when pancreatin solution without renal extract was incubated, or when acetone was added to an unincubated mixture of renal extract and pancreatin, and appeared to be formed by the process of digestion (Fig. 4, *a*, *b* and *c*). It was found that the potency of this material was not diminished by boiling (Fig. 4, *d*), that its effect came on more rapidly, was more pronounced and disappeared more quickly than did the effects produced by the injection of the protein-like pressor substance produced by the previous precipitation methods (Fig. 4, *a*, *d*, *g* and *h*). The pressor response was less immediate and of longer duration than that produced by adrenalin (Fig. 4, *a* and *f*); on the other hand, the response of the blood pressure was identical in character to that produced by the injection of tyramine (Fig. 4, *a* and *e*). In each case the pressor response reached its peak in 2 to 4 minutes and lasted 6 to 8 minutes.

As illustrated in Figure 5, added tyramine could be quantitatively recovered by the procedure used in isolating the pressor substance from the digested mixtures. It has been shown by Tainter and Chang[20] that the pressor effect of tyramine can be abolished by the previous administration of cocaine. This effect is not specific for tyramine.[3] The pressor substance obtained from the digested renal extract was found to be reversed by a preliminary injection of cocaine (Fig. 6, *d*), whereas the pressor response of the other fractions described above was unaltered. Since there is no reason to suspect the presence in tissue digests of pressor agents—other than tyramine-like substances—which are inhibited by cocaine, these observations suggest that the pressor agent in the digested renal extract is a substance with properties similar to those of tyramine, that it is

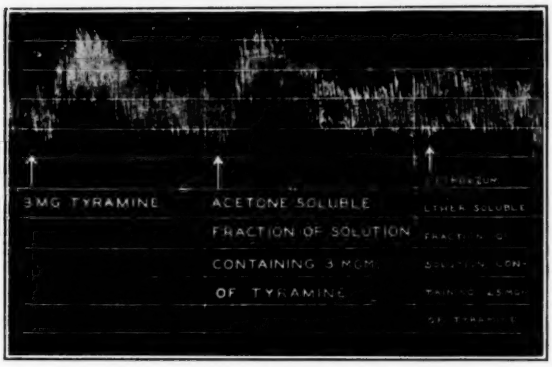
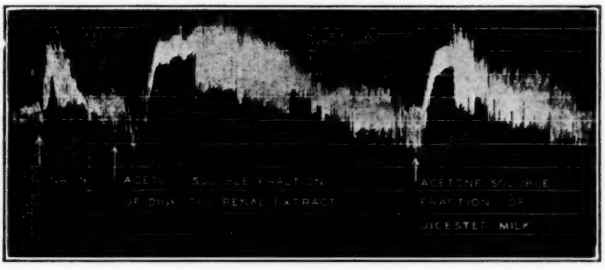


FIG. 5.—A solution of tyramine comprising 3 mg. of tyramine per cc. was treated similarly to digested renal extract, *i. e.*, with acetone and with petroleum-ether. The figure shows that the tyramine was recovered quantitatively from the acetone fraction; the petroleum-ether fraction having a negligible pressor effect. (The distance between two adjacent horizontal lines indicates 20 mm. of mercury.)

BEFORE COCAINE



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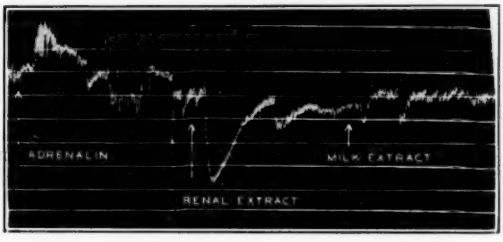

FIG. 7.—The figure shows that the pressor effects obtained from digested milk and from digested renal extract were abolished by cocaine; that of adrenalin was not. (The distance between two adjacent horizontal lines indicates 20 mm. of mercury.)

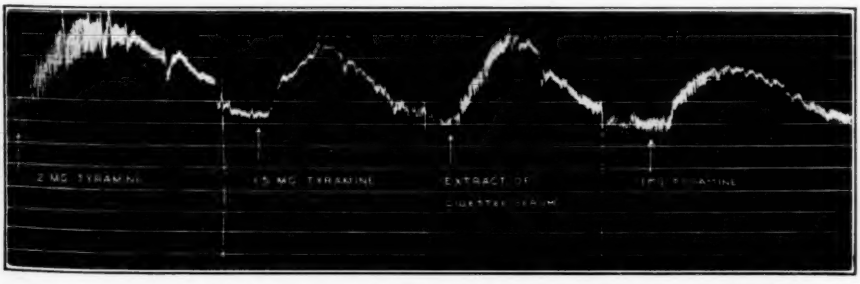

FIG. 8.—The injection of extract of digested pig serum (the amount indicated corresponding to 5 cc. of serum) gave as great a pressor effect as did 1.5 mg. per kg. of body weight of tyramine. (The distance between two adjacent horizontal lines indicates 20 mm. of mercury.)

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produced in the process of digestion, and that it is not responsible for the pressor effect of the fresh renal extract.* The blood pressure raising principle in the latter appears to be dependent on an entirely different substance of a protein-like nature, which exists preformed in the extract, is precipitable by the methods described, and appears to correspond with Tigerstedt's "renin;" the former substance seems to be the pressor agent which develops after several hours' digestion of renal extract, and is probably the pressor principle

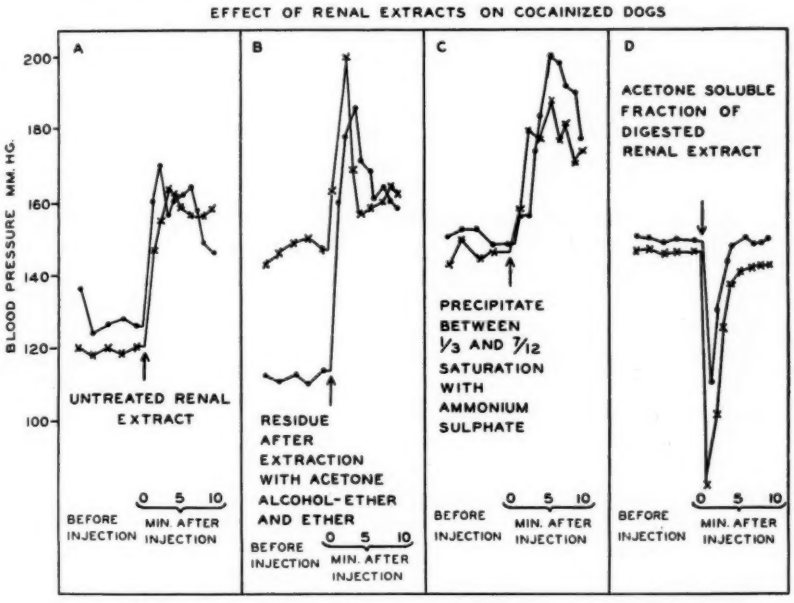

FIG. 6.—The previous administration of cocaine did not prevent the pressor effect of fresh renal extract, of renal extract precipitated by organic solvents or of renal extract precipitated by ammonium sulphate. However, cocaine did prevent the rise in blood pressure produced by the acetone-soluble fraction of the digested renal extract.

occurring in such extract after prolonged autolysis. A series of observations were made on extracts which had been rendered sterile by filtration or by the addition of disinfectants. The results obtained were inconclusive and we are not entirely certain whether the pressor substance can be produced by digestion in the absence of bacterial growth.

Extracts of Other Tissues. In order to determine whether the pressor principles obtained by these various methods were specific for renal tissue, similar preparations were made from other organs (Table 1). It is seen that precipitation with acetone or with

* Further efforts to identify this substance as tyramine by the Gerngross reaction, and preparation of tyramine di-benzoate were not attempted.

TABLE 1.—EFFECT ON BLOOD PRESSURE OF ORGAN EXTRACTS.

Method of extraction.		Organ from which extract was prepared. Liver.	Spleen.	Lung.	Heart.
Precipitation with acetone, alcohol-ether and ether	Number of tests	3	4	3	4
	Number of times blood pressure increased*	0	0	0	0
Precipitation with ammonium sulphate	Number of tests	4	4	6	0
	Number of times blood pressure increased*	0	0	0	0
Digestion and extraction	Number of tests	5	3	3	2
	Number of times blood pressure increased*	4	3	2	0

* Increases of less than 20 mm. of mercury were considered as negligible in compiling this table.

ammonium sulphate of the saline extracts of liver, spleen, lung and heart did not yield a pressor substance. However, when saline extracts of liver, spleen and lung were digested, an acetone-soluble powerful pressor agent was obtained which was similar in all respects to that obtained from the kidneys. Application of the same technique to milk and white beans gave positive results in each of 3 instances. On the other hand, negative results were obtained from heart (2 tests), Jack beans (3 tests), sweet potato (2 tests), gelatin (2 tests), egg white (2 tests) and corn meal (1 test). The pressor effect obtained from substances which gave positive tests was also inhibited by cocaine (Fig. 7).

In a number of instances pig serum was digested with pancreatin and also yielded a powerful pressor substance similar to that obtained from digested renal extract (Fig. 8). This pressor effect was not obtained from unincubated serum with or without pancreatin, and was not secured from serum incubated without pancreatin (Fig. 9).

Discussion. The foregoing observations indicate that there can be obtained from renal extracts, 2 different pressor substances. One of these appears to be a protein, having some of the properties of pseudoglobulins. Its action comes on slowly after intravenous injection, the pressor response to it develops slowly and is quite prolonged. This substance corresponds to that designated as "renin" by Tigerstedt and Bergman.[22] The other pressor agent is not present in fresh renal extract but can be produced by digestion. It has chemical, physiologic and pharmacologic properties which insofar as they have been tested are similar to those of tyramine. This latter substance is not specific for kidney tissue, but can be obtained from digested extracts of various organs and of certain vegetables. It can also be obtained from digested serum.

One is naturally interested in knowing whether the 2 compounds under discussion are of any significance in hypertensive states. On this question the experiments reported in the present paper throw no direct light. However, Harrison, Blalock and Mason[9] and

Friedman and Prinzmetal[6] showed that fresh saline extract of kidneys rendered ischemic by the application of a Goldblatt clamp to the renal artery usually had a more pronounced pressor effect than did similar extracts of the opposite normal kidney of the same dog. These findings suggest that "renin," which appears to be responsible for the pressor property of untreated renal saline extracts may be

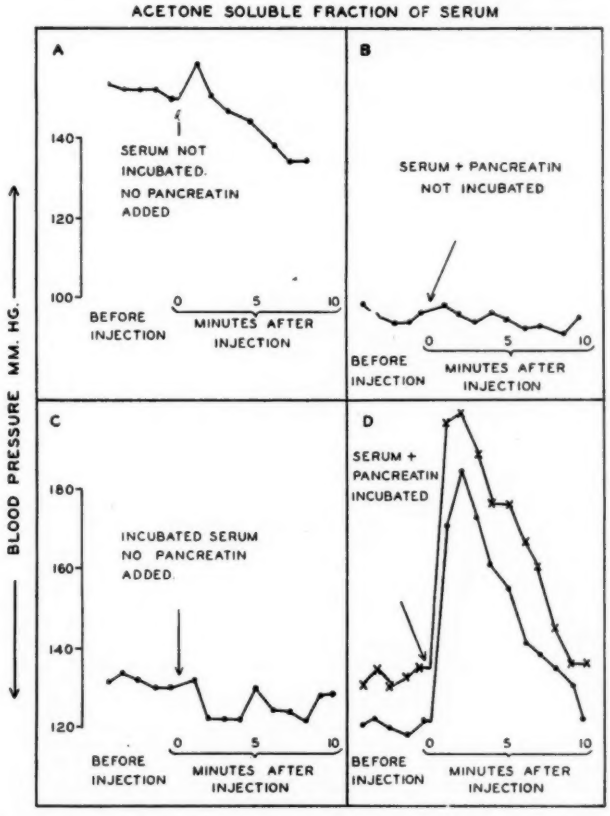

FIG. 9.—Acetone extracts of fresh serum (*A*), fresh serum plus pancreatin (*B*), and of serum incubated without pancreatin (*C*), were not pressor. However, incubation of a serum to which pancreatin had been added yielded a potent pressor substance (*D*).

of some importance in the production of the increase in blood pressure displayed by the animals with renal ischemia produced by applying a Goldblatt clamp.

Whether tyramine or tyramine-like substances are of any significance in the production of hypertension is uncertain. Some authors believe this to be the case;[17,23] others hold the reverse view.[19] Our own experiments only emphasize again the readiness with which

they form under condition of protein degradation. The condition of the method of preparation which we have used, *i. e.*, pancreatin digestion plus bacterial action, could exist only in the intestinal tract. It would seem that observations concerning the formation of pressor substances in the gastro-intestinal canal and concerning the ability of the liver or other organs to remove them from the portal blood in states associated with hypertension might be useful. The possibility of the formation of tyramine in other sites in the body should also be investigated.*

Summary. 1. A pressor principle can be separated from fresh saline extracts of renal cortical tissue by ammonium sulphate precipitation. The properties of this substance are those of Tigerstedt's "renin," and it appears to be responsible for the pressor effects of fresh untreated saline extract of kidneys.

2. A pressor principle can be extracted from the acetone-insoluble fraction of renal extract by appropriate treatment. This principle has certain properties resembling those of "renin," but it is not known whether the two are identical.

3. Autolysis or digestion of renal extract results in the appearance of a pressor principle which has some properties of tyramine.

4. Evidence is submitted that the pressor effects of fresh saline extract of kidney is not due to tyramine, or a tyramine-like substance.

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* Since this paper was prepared for publication further investigations have yielded two additional points of importance. 1, The rat, anesthetized with sodium pentabarbital, is a more satisfactory test object for these extracts than is the unanesthetized dog; 2, for use in the rat the most satisfactory method of preparation of the renal pressor substance corresponding to "renin" is precipitation with alcohol, and reëxtraction of the pressor body from this precipitate with water. The details of these experiments will be submitted in a later publication.

MARIHUANA.

Our New Addiction.*

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With lurid and startling headlines, the public press has announced: "Spread of Marihuana Held Menace to Country's Youth." "The Fight Against Hashish of America." "Reefer Peddler Held in $15,000.00 Bail." "Marihuana Spreads Its Net in America." "America Menaced by New Dope Evil." "Marihuana —Assassin of Youth." "Sex Crime Epidemic Laid to New Dope."

Marihuana, spelled different ways, is not the proper name of the drug, its addiction is not new, except with us, nor are the effects of indulgence so baneful as the foregoing headlines would indicate. Nevertheless, during the last 10 years, its excessive use had become sufficiently prevalent to cause all states except North Carolina and Tennessee, to enact legislation against its distribution. Finally, the Federal government regarded the situation so serious that control of the growth of the plant was sought through the imposition of certain excise taxes, which resulted in the enactment of a bill at the last session of congress, giving marihuana a separate listing among narcotic drugs, a law that actually became effective October 1st of this year. The distribution of this plant is so widespread that, in addition to its cultivation, it may be found growing wild along the roadsides and upon vacant lots in many states, particularly in the west. Even Philadelphia County has not escaped. In San Quentin, California, and in the Colorado State Reformatory, it was found growing within the prison walls, where it was being harvested and smoked by some of the inmates. Since a number of marihuana's products have important legitimate, commercial uses, enforcement of this law may be attended by some of the difficulties encountered during our Prohibition era. With us, the proper name of the plant is cannabis sativa; cannabis, cannabis indica, Indian hemp and hashish are synonymous terms. Marihuana is a mongrel, Mexican word, meaning pleasant feeling. In underworld argot, the drug is known by many different names, such as, "Indian hay," "greefo," "mu," "motta," "mooter," "reefer," and "Mary Warner" is marihuana personified.

Historical Survey. We are repeatedly told that from the Arabic, "Hashishan" (herb-eaters), comes our word, assassin, though this is questioned by the biographers[5] of Omar, that tent-maker of old. In Persia, long before the Christian era, there lived a military and religious sect known as the Assassins, who were sometimes incited to greater violence in their atrocities, by the use of hashish, which is the historic name of marihuana. The plant had its origin in the

* Read before the Philadelphia Neurological Society, November 19, 1937.

East and preparations made therefrom were either smoked, chewed or drunk. Homer told of the power of hashish to afford solace in our moods of distress, as well as of its more harmful effects. The growth was cultivated in China three centuries before Christ; writings of the Assyrians, Greeks and Romans, all speak of familiarity with its use, and in Sanscrit was recorded the popularity of "Gaiety Pills," made from hemp and sugar. About 1800, when Napoleon's army was in Egypt, he found it necessary to prohibit its use by his soldiers. In addition to smoking hemp, the natives of India prepare from the powdered leaves a beverage called "bhang," and a similar drink of the Greeks was "nepenthe." In Egypt, now as in ancient times, it has many devotees; elsewhere in northern Africa, the habit is a powerful one, hashish often being preferred to alcohol or opium. At times, the drug has played an important part in religious observances. From India, it spread to Mongolia. Both hashish and opium are charged with having caused the Malayan to run "amok," but those murderous episodes often occurred without such drug domination. In time it appears the plant was introduced from the eastern hemisphere to Mexico and from there spread to the United States. Our early settlers, finding its fiber useful, began its cultivation.

The Plant and Its Use. This hardy, annual growth thrives in most climates with little cultivation. The plant is slender with an erect, thick, stringly, angular and branching stem, varying in height from 3 to 16 feet; the leaves which grow alternately and for the most part point downward, are formed of from 5 to 7 blades with saw-toothed edges. Considerable commercial value attaches to the plant. From its fiber, twine, rope, bags and clothing may be made. When ripe, the small, whitish flowers at the top of the female plant are found to yield a sticky, greenish, resinous substance which contains the active principle of marihuana; however, recent research by Munch, showed definitely that the substance may also be obtained from the male plant. Botanists tell us that scientifically, the seed is a fruit. From this seed, a rapidly drying oil may be extracted, useful as an ingredient of paints and varnishes; the oil also finds use in the manufacture of soap and linoleum. The seed is an important food for pigeons, acting as a general tonic and unexcelled in maintaining a healthy condition of their plumage; doubtless seed spilled from their feed boxes is responsible for some of the patches of marihuana found growing wild. It is said application of heat and moisture soon destroy all power of germination, so that an easy method of sterilization appears to be available. In the Orient, when food becomes scarce, the seed is ground and eaten as we eat oatmeal. Therapeutically, marihuana's preparations have been used for the relief of pain, in migraine, to produce a euphoria and to overcome nervousness; but the drug has mostly fallen into disuse, partly owing to its greatly varying degrees of

potency. John Stuart Mill, philosopher, wrote of its power to revive forgotten memories, and in my inquiries, smokers have frequently informed me that while under its influence, they are able to recall things long forgotten. If through such use, the unconscious mind could be rendered more accessible, possibilities as an aid in psychoanalysis and psychotherapy are shown.

Effects of Excessive Use. The active principle of the plant, cannabinol, chiefly affects the cerebrum and excessive use results in what is known as cannabinism. While portions of the plant may be chewed or beverages made therefrom, with us, indulgence for the most part is through cigarettes, known in slang as "reefers;" a reefer is made of brownish paper, shorter and thicker than the common cigarette, and in price ranges from 10 cents each to 2 for 25 or 35 cents. The smoke which is always inhaled, is retained much longer than is the custom in cigarette smoking. The odor given off is said to be rather distinctive. Though varying greatly in strength, 2 or 3 reefers usually have the desired effect. The product may also be purchased in bulk—a mixture of most parts of the plant. Extent of effect depends upon potency of the plant, care in curing the product, individual susceptibility and the quantity used. Often, the first noticeable change is a lessening of the subject's power fully to control his thoughts and actions. Distractibility is shown by rapid interruptions in the continuity of thought and by the many disconnected ideas that thrust themselves into the field of consciousness. In addition to a feeling of exhaltation and euphoria, the smoker is aware of increased power and energy. By a determined effort of the will, he is for a time able to withdraw from his reveries, but if there be further indulgence, such possible lucid intervals become shorter and less frequent. Illusions are common and faces of others often take on grotesque expressions. Imagination may be stimulated to the extent of delirium, sometimes with fascinating hallucinatory creations appearing; these may be pleasing, ludicrous, gruesome or sensual. Inhibition being much reduced, dominant emotions become magnified and thus, as is often observed, no two persons are similarly affected; the genial man grows more fond of his fellows, the quarrelsome one pugnacious, the timid one more fearful and the criminal is emboldened. Exaggeration is the rule, so that the plainest food may taste delicious and hearing may be painfully intensified. Perception is disturbed in the matter of time and space; minutes seem hours and hours days; to pass over a twig a long step or a jump may be taken. Excitomotor activity is often present and a reckless abandon that may extend to violence. Sensations of pain and touch are diminished and the pulse rate is increased. With the full return of consciousness, no subsequent ill effects are experienced. Marihuana is a diuretic and its use induces hunger. Since the craving that accompanies alcohol and morphine addiction are not present, indulgence can be easily discontinued. There are

no known fatalities. Marihuana is said to be a source of inspiration for some writers, painters and other artists. Of one whose confidences he received oftener than he desired, Ribot[4] said: "I once knew a man who for 20 years constantly took hashish in large doses; he withstood the drug better than might be expected, but finally died insane." The French poet, Baudelaire,[6] a member of the Hashish Club in Paris, used hashish and wrote about it; he also used alcohol and opium, and had a coarse, insatiable, colored mistress; he contracted syphilis before 20 years and died at 46, according to Lombroso, of paresis; but from the description, it appeared more like another form of syphilis; however, it seems unlikely that hashish was more than contributory to his end.

Lewin,[2] the Berlin authority on narcotics, divides eaters and smokers into three groups: 1. Exhibit a state of general euphoria and excitement with hallucinations and illusions even extending to deliria, less aggressive, however, than that of alcohol. They may be irresponsible for the time being. A cure may usually be effected in a day. 2. An acute mania develops. The patient is agitated, garrulous, suffers from insomnia, there are sense illusions, delusions of persecution and sometimes a violent fury. The condition may last months and is not always curable. 3. A group of mentally weakened individuals who pass into a maniacal state after every excess of hashish; they are overtalkative, lazy, but the slightest provocation may evoke a violent state. After dismissal from the hospital they relapse, become agitated, insult their friends and easily show violence. Many such chronic subjects are incurable and end in dementia. Individuals of this group seldom commit crimes. Generally speaking, it is said the sudden and rapid cure of such diseases, is the only diagnostic sign of insanity due to hemp. In Bengal, of 232 cases of mental disease, 76 were caused by hemp, only 34 of which were cured. In Cairo, out of 248 inmates, 60 men and 4 or 5 women owed their mental disease to hashish. But these statistics are from lands where indulgence is very extensive and has been carried on for generations. Lewin believes it probable that the craving for hemp may be inherited. As to its being an aphrodisiac, he states: "Perhaps sexual potency is increased at the outset, but it nevertheless diminishes during the subsequent addiction of the drug, as is the case of opium smoking."

Those of us who were students of H. C. Wood, the elder,[8] will recall the vivid description by him of his personal experience after taking a large dose of cannabis indica extract. The first manifestation was noticed several hours after, while in the house of a patient during the writing of a prescription; he seemed entirely oblivious of his surroundings; then, catching himself, he apologized for what to him seemed a tremendously long time, but the patient assured him he had only been a few minutes. Returning home, he found himself somewhat excited and with an inward feeling of joyousness. Physical fatigue was banished and idea after idea raced through

his mind. Self-control was lost, he laughed and made funny gestures. His legs felt numb, his mouth dry, the pulse was 120, and becoming alarmed, he summoned a fellow practitioner; seeing the physician approaching, Wood observed that he seemed a vast distance away and a corresponding time coming toward him. His legs felt heavy and pinching them caused no pain. There were periods when he seemed unconscious though it was possible for him to be aroused. A disturbing feeling of personal antagonism between himself and his will power was experienced. Double consciousness was present—a feeling that he was himself and someone else at the same time. There were no hallucinations and no aphrodisiac reactions. Urinary secretion was markedly increased. About 8 hours after ingestion, he went to bed and wakened in the morning with a clear mind, though soon after, and from time to time during the day, there were brief fragmentary experiences similar to those of the night before. Except for a marked anesthesia of the skin all day, there were no unpleasant after effects.

In the Eastern State Penitentiary we have many colored prisoners, some of whom had been thoroughly familiar with reefer smoking as well as with the atmosphere where provision was made for such indulgence. However, in none of these prisoners had there been any evidence that marihuana was a factor in their crimes. One prisoner who had been a periodic smoker for 17 years, was an adept at curing the product which he stated was similar to that used for tobacco. Another who was particularly informative, had been proprietor of a "club," "den," or "tea-pad," slang for places where reefer smokers congregate. It is said such clubs exist in most large cities. One of the more expensive of these was described as a room sufficiently large to accommodate two dozen devotees, with smaller adjoining rooms for their more intimate relations. The smokers were usually dressed in silk pajamas and would sit or recline upon the many silken pillows disposed about the floor; upon the walls were hung pictures and other objects, mostly of women, chosen for their sensuous or sensual appeal. Such rooms were purposely unventilated, and an initiate, entering the heavily charged atmosphere, would soon come under the influence of marihuana. The lights are low, which is more conducive to reveries and perhaps to mating. Music is softly played, though by way of diversion, coming from a phonograph, may be heard the sonorous and sensual voice of Cab Calloway, singing "That Funny Reefer Man." It is said the acts of many such entertainers is preceded by the smoking of reefers. Various slang terms are used by addicts, the more common ones being, "in," meaning the subject is under the influence, and "out" which is the reverse; "high," is the state desired to attain and to maintain for some time; this "high" effect is present when some object that is being observed, grows smaller and smaller, until only a blurred spot remains. The experienced smoker is able to go "in" and "out" at will. Reactions vary with different individuals; they may talk, many

laugh, sing, dance or sleep; most of them are happy, some are quarrelsome and upon little or no provocation become assaultive; many prefer to sit or recline alone, given over to their reveries; most objects seen are reduced in size and time is greatly lengthened. A few, becoming grandiose, have but to desire something, when seemingly, it is realized—wish-fulfillment not Freudian in origin. The ability to recall in detail things long forgotten, was frequently told me. Any sudden shock has a sobering effect; or, if it is desired to come rapidly out of the "drunk," taking copious quantities of cold water, or a cold bath, together with an abundance of fresh air, will soon restore the addict. These colored subjects insist that marihuana is an infallible remedy for toothache.

Relation to Crime. Homicides, suicides and assaults, particularly those upon sex, are among the more grave indictments of marihuana. Perhaps the most lurid and frequently repeated case is that of a Florida youth, who, while said to have been under the influence, imagined some people were going to cut off his arms and legs; whereupon, in a frenzy, he seized an ax, killed his father and mother, 2 brothers and a sister. One man, greatly to his horror when coming from under the influence, learned that he had decapitated his best friend. Still another, becoming enraged at his wife, killed her in the street before many witnesses. A high-school girl had heard some whisperings concerning the "kick" offered by the new kind of cigarette; after smoking a reefer, she was able to dance long without fatigue; time became elastic and in her state of exhilaration all else seemed inconsequential; studies were entirely neglected, she became despondent, but a few puffs on her faithful cigarette brought relief; finally, coming to full realization of her school problem, she deliberately walked to an open window and leaped to her death. Thus, says the report, did marihuana solve her problem. These cases and similar ones have been very incompletely reported, since we do not know what other factors may have entered into their acts of violence.

By way of contrast, the following references are given: In 1932, this announcement was made by the State Narcotic Committee of California: "The sensational statement that peddlers are selling narcotic drugs to minors cannot be substantiated by our records." From the archives of the International Narcotic Education Association, there were reported 8382 problem children, from 28 typical American communities; of these, 29 were reported as drug addicts, but 26 were only using snuff, one little girl had used headache powders, a boy had purchased a drugged cigar, and a third case was of unknown nature. At the Bellevue Hospital, Bromberg[1] found in 361 subjects classed as psychopathic personalities, 32 were drug addicts, only 7 of whom had smoked marihuana for any considerable time, and none of the assault cases investigated, "could be said to have been committed under the drug's influence." As observed in his experience, "the anti-social, aggressive and

sadistic elements of the personality uncovered by the drug are responsible for the crime rather than any specific crime-producing properties of marihuana." Bromberg further adds: "It is probable that alcohol is more responsible as an agent in crime than marihuana."

Perhaps the most extensive use of the drug is found in New Orleans where, in 1934, it was said one out of every four persons arrested was a victim of the drug. Eugene Stanley,[7] district attorney of New Orleans, in speaking of the difficulties experienced in determining the degree of responsibility in one under the influence of marihuana says: ". . . while some of these experts are conversant with the nature and effect of the drug, it has been the experience of the author that many psychiatrists know nothing whatsoever of the effect. . . ."

Summary. Known in history as "hashish," and in a recent congressional act as "marihuana," *cannabis sativa* grows so widespread in this country and has such important commercial uses that law enforcement may be hampered. From marihuana's fiber, twine, rope and bags are made; it yields a rapidly drying oil, useful as an ingredient of paints and varnishes and in the manufacture of soaps and linoleum. Birds thrive on the seed.

Therapeutically, marihuana has been employed for the relief of pain, in migraine, to overcome nervousness and to produce euphoria; but the drug is now seldom used, owing to its greatly varying degrees of potency. Its use causes things long forgotten to be recalled in detail, an effect that invites careful research. Preparations of the plant are smoked, chewed or drunk, but in this country indulgence is mostly through cigarettes, known in slang as "reefers."

Marihuana is not a habit former like opium and cocaine; from a so-called "drunk," there are no hangover symptoms and there are no known fatalities.

But even moderate use is not without danger—its well known action on perception, the lengthening of time and space, could prove most disastrous if, for instance, the smoker were driving a car. When used daily in large quantities, the direct action upon the cerebrum often causes chronic mental deterioration, an effect that has been known for ages in India and Egypt.

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THE STERILITY OF ALCOHOL.

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Forty years ago, Minervini[15] and later other workers demonstrated that non-spore-forming organisms could be killed by alcohol. Diluted alcohol was found to be more effective than alcohol of high concentrations, that of 70% strength being the most efficient. Minervini using the thread method noted that while a 70% alcohol killed the *Escherichia* (Bacillus) *coli* and *Staphylococcus aureus* in 60 minutes, dehydrated or absolute alcohol required at least 12 hours for the former and more than 3 days for the latter organism before destruction was complete. Olitsky and associates[18] and others, however, have reported that the addition of alcohol to body fluids results in the production of dense coagula which protect microorganisms and prevent penetration by the alcohol.

The inefficiency of alcohol as a germicidal agent for spore-forming bacteria has been demonstrated repeatedly. In 1881, Koch[11] showed that neither dilute or strong alcohol would kill anthrax spores in 110 days. Minervini[15] and Russ[20] confirmed Koch's work. Stokvis[24] revealed that *B. megatherium* remained viable for 2 weeks in alcohol. Heim[9] was able to cultivate *anthrax bacilli* from infected threads which had been immersed in alcohol for 20 years. Dozier[6] found that alcohol possessed no bactericidal effect upon the spores of *Clostridium botulinum*. Nye and Mallory[17] reported the inefficiency of alcohol upon the spores of *Clostridium welchii* in a routine procedure of disinfecting surgical instruments, such technique having resulted in a serious outbreak of infections following operations. Schmidt,[23] in an investigation of catgut, reported that the sterile catgut threads become infected by alcohol when using terminal sterilization. Coulthard and Sykes[3] reported that vegetative forms of bacteria are destroyed in a few minutes by concentrations over 60% but ethyl alcohol was impotent against bacterial spores.

In practice today, no reliance is placed upon the use of alcohol (dilute or strong concentrations) as a bactericidal agent for spore-forming organisms. If used at all, it is employed only against the weak resistant organisms and even here considering the technique when it is used (exposures of less than 5 minutes), the alcohol itself in all probability exerts little or no effect as a bactericidal agent.

Alcohol Employed Parenterally. Alcohol in various concentrations has been used externally; internally it has been consumed (by mouth) as an ingredient of various medicinal preparations or of liquors. More recently, however, parenteral therapy with alcohol has been advocated as an aid in the relief of pain and discomfort in a variety of abnormalities. Dogliotti[5] employed subarachnoid injections of absolute alcohol for the relief of peripheral pain in the lower part of the back, the pelvis and legs. Gilcreest and Mullen[8]

advocated epidural and transsacral injections of alcohol for the relief of pain. Ruth,[21] Condamin and Arnulf,[2] Davis,[4] Newman,[16] Saltzstein,[22] Baker[1] and many other workers have employed parenteral alcohol therapy with apparent success. More recently, Pozzi and Belleli[19] employed intravenous injections of alcohol (10 cc. of 33% alcohol in a 45% solution of dextrose) and they noted an increase in the bactericidal power of the blood. Meynier[14] discusses the intraspinal (intrathecal) injections of alcohol for intractable pain in the pelvis and lower extremities.

Bacteria in Alcohol. Commercial alcohol has been generally accepted as bacteria-free. Only those workers who concern themselves with problems in disinfection and sterilization were familiar with the actual bactericidal properties of alcohol and had knowledge of the bacterial content of commercial alcohol. With the advent of the more frequent use of alcohol as a disinfectant in surgical procedures and its recent use for parenteral administration, several articles appeared in foreign publications concerning the germ content of commercial alcohol. Many devices and methods of sterilizing alcohol have been described.[13] Even in our own country some manufacturers have marketed sterilized absolute alcohol in ampules to be used for parenteral administration. Recently Kuhn and Dombrowsky,[12] Knorr,[10] and also Eschenbrenner[7] reported on the finding of bacteria, in particular spore-formers, in commercial alcohol. My attention was especially directed to the fact that Knorr[10] found more than half of the 38 samples of commercial alcohol (of different concentrations) laden with sporulating organisms. An examination of the American literature did not record findings of the bacterial content of commercial alcohols used in this country.

Experimental. A survey of the literature and personal conversations with many workers who employ injections of alcohol revealed that at least in this country the alcohol used in such injections is rarely redistilled, Berkefeld-filtered or otherwise treated as in sterilization techniques. Not one case of infection has come to the attention of the workers with whom I spoke, even though a total of several thousands of such injections were given by them. The alcohol used generally is the 95% (by volume) strength or the absolute product (dehydrated alcohol) obtained directly from the regular stock bottle in the drugstore or clinic, and the amount employed in each injection varies from 0.2 to 2 cc., the dose being usually 0.5 to 1 cc.

Accordingly 125 samples of commercial alcohol, consisting of 100 samples of 95% and 25 samples of absolute (dehydrated) alcohol were obtained. The samples were collected from retail pharmacies; from the drugstores, clinics, pathologic laboratories and operating rooms in large and small hospitals; from the bacteriologic, pathologic, chemical, operative pharmacy and botanical laboratories in teaching institutions; from containers in the offices of medical practitioners; and from stock cans, carboys and drums. The 25 absolute

alcohol samples, though collected from 23 different places, were in all instances present in 1-pint cork-stoppered bottles. The 95% alcohol samples were obtained from all kinds of containers kept under varying conditions. There were small and large bottles, filled and partially filled, cork and glass-stoppered (some were not even stoppered), carboys, barrels and metal drums.

From each of the 125 samples, 1-cc. and 2-cc. portions were cultured aërobically, using at least 50 cc. of meat-infusion dextrose broth for each 1 cc. of alcohol to be cultured. A 2% alcoholic solution is not bacteriostatic and all kinds of spore-bearers were grown with ease in media of this alcoholic content. All cultures were incubated aërobically for 2 weeks at 37° C. and then for another 2 weeks at 20° to 22° C. Of the 125 samples, 10 of the absolute alcohol and 50 of the 95% alcohol samples which were cultured as above were also inoculated and incubated under anaërobic conditions. After 1 month incubation, none of the 125 samples revealed growth in the above medium when cultured in portions of 1 and 2 cc.

The following experiment revealed that spore-bearing bacteria could survive in 95% alcohol for varying periods of time. One-tenth cubic centimeter of a 1-week broth culture of *B. subtilis* was added to each 30 cc. of 95% alcohol placed in 6-ounce cork-stoppered bottles. A broth culture of *B. megatherium* was added in the same proportion to another bottle containing alcohol. Two 6-ounce sets were made, using two different strains of each organism, one in each instance as a check. The containers were stored at room temperature. At intervals of 3 to 4 days, 1-cc. and 2-cc. portions of the alcohol containing the added cultures were inoculated into meat-infusion dextrose broth, using at least 50 cc. of medium for each 1 cc. of alcohol cultured. The *B. megatherium* was found to survive in the 95% alcohol for a period of 88 and 96 days respectively, while 215 and 276 days respectively were required for the destruction of the two strains of *B. subtilis*.

Summary. One hundred and twenty-five samples of commercial ethyl alcohol, consisting of 100 samples of 95% alcohol and 25 samples of absolute alcohol, were obtained on the open market from as many different sources as possible. All of these, samples of ethyl alcohol produced in this country, were found to be free of bacteria and their spores. This is in contrast to reports from Europe where the commercial product was found to be contaminated frequently with spore-formers.

Alcohol (95%) did not kill *B. megatherium* and *B. subtilis* until after approximately 3 months' exposure in the first instance, and 7 and 9 months respectively were required for the destruction of the two strains of *B. subtilis* used in this experiment.

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NEGATIVE RESULTS OF RHUS ANTIGEN TREATMENT OF EXPERIMENTAL IVY POISONING.

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Rhus toxicodendron antigen intramuscularly and the tincture by mouth are frequently used in the treatment of ivy poisoning. Williams and MacGregor,[4] Strickler[3] and Bivings[1] have written encouraging papers on both prevention and treatment with these preparations.

Krause and Weidman,[2] however, in experiments on human volunteers, concluded that the preventive system of treatment of Strickler did not "prevent." They believed that the beneficial results which had been reported were dependent on and ascribable to the variable susceptibility of different individuals and the varying intensity of the irritant as applied at different times. They observed that transient pruritus ani appeared in the majority of those receiving preventive treatment. They were convinced that the pain at the sites of intramuscular injections outweighed any possible benefit in reducing the danger of future attacks of ivy poisoning. This paper deals with the therapeutic use of the antigen.

Methods and Materials. The plants employed for producing the dermatitis were identified as Rhus toxicodendron by the department of Botany of the University of Pennsylvania. A tincture was made by soaking the leaves, bark and stems for several days in pure grain alcohol. A 1 inch square of sterile gauze saturated with this tincture was applied to the skin for 24 hours. To prevent spread the area was covered with adhesive tape. Controls on the same subject were made, using a similar patch test with the pure alcohol.

The antigen used was supplied fresh by one of the large drug companies. The preparation and method of administration was that advocated by Strickler.[3] As recommended, 0.5 cc. was given intramuscularly; 24 hours

later a second injection of 1 cc. followed; subsequently, at 48-hour intervals, one or two injections of 1 cc. were given.

Of 29 volunteers (medical students), 23 reacted with a typical eruption. Of these, 14 were observed as controls, while 9 received treatments. For uniformity, treatment was instituted as soon as vesiculation began to appear. Detailed records of each case were kept on uniform protocols, and conclusions as to results were made only after statistical analysis of the data.

Results. In the control group the duration of the dermatitis ranged from 3 to 16 days (average, 9 days). In the treated group the average duration was 11 days and in none had the symptoms entirely disappeared in less than 9 days. As for itching, it occurred in all cases and lasted in the control group for 3 to 4 days; in the treated group 3 days was the average, even omitting the solitary severe case with generalized pruritus of 9 days' duration.

The majority of the volunteers experienced painful reactions at the site of injections, lasting from 2 to 48 hours. Among these students the consensus was that those who received treatment experienced the greater discomfort. In 1 individual, nausea followed after each injection. Generalized pruritus occurred in one who had received 3 injections.

Summary and Conclusions. 1. Rhus antigen was tested for curative power on 9 volunteers, 14 acting as controls.

2. The antigen injection intramuscularly at the onset of the eruption did not appear to shorten the course of the dermatitis. Its effect on the intensity of the pruritus was slight, if any.

It appears, then, that the routine use of the antigen treatment of ivy poisoning is not warranted. The discomfort and expense, upwards of $3.00 for 4 injections, overbalance the very questionable therapeutic effectiveness.

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THE URINARY OUTPUT OF VITAMIN C IN ACTIVE TUBERCULOSIS IN CHILDREN.

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Since 1928 when Szent-Gyorgyi[3] isolated hexuronic acid from the cortex of animal adrenals, brilliant investigations have added much

to our knowledge of vitamin C. Its chemical structure and properties, now well known, have recently been reviewed in detail by Wright and Lilienfeld.[13] While the part played by vitamin C in human physiology is still incompletely understood, it is definitely known that it is essential for the maintenance of normal capillary permeability. Wolbach and Howe[12] in their studies concluded that a lack of vitamin C results in an "inability of the supporting tissue to produce and maintain intercellular substance." They further postulated that this defect also holds true for the connective tissue of the teeth. Schroeder[7] is of the opinion that hypovitaminosis C prolongs the coagulation time of the blood, diminishes the number of blood platelets and causes a decrease in the hemoglobin. Among positive effects attributable to the vitamin are improvement of appetite, and stimulation of growth and glandular functions. More recent investigations suggest that it is also involved in defensive mechanisms against bacterial toxins.

Studies of the rôle of vitamin C in infectious diseases are comparatively few. However, sufficient data have accumulated to suggest that a deficiency may occur in various infectious states. Wilkinson and Ashford[11] found this in 3 cases of Addison's disease and point out a possible relationship between vitamin C deficiency and pathologic pigmentation. The deficiency they observed in general hospital cases was attributed to an inadequate intake of the vitamin. Harris *et al.*[6] believe that hypovitaminosis C may occur in any type of pyrexia and toxemia. Abbasy *et al.*[2] found acute and chronic juvenile rheumatism associated with a vitamin C deficiency and state that these children require a greater amount of the vitamin in their diet than do normal children. Grunke and Otto[4] found that diphtheria was always associated with a low urinary excretion of ascorbic acid. They made similar observations in scarlet fever, measles, bacillary dysentery, paratyphus, erysipelas and colitis. However, the upper level of what these investigators consider low elimination figures (15 to 20 mg. /24 hours) falls within the normal excretory level of most workers.

Since many investigators have found varying forms of hypovitaminosis C associated with toxemias, it is reasonable to expect a similar occurrence in active tuberculosis because of its chronicity and prolonged toxicity. The literature presents many suggestions of vitamin C deficiency in tuberculosis, but there is little actual evidence of studied cases. Schroeder[7] believes that tuberculosis is associated with increased vitamin C metabolism, as manifested by decreased elimination of ascorbic acid in the urine and Widenbauer[10] made similar observations. In an attempt to obtain further data, the authors have routinely determined the urinary output of all cases of active tuberculosis in the children's wards of the Tuberculosis Service of the Buffalo City Hospital.

Method. The 24-hour urinary output of vitamin C was determined by a modification* of the dichlorophenolindophenol technique of Harris and Ray.[1,5] From 5 to 15 cc. of the 24-hour sample is brought to a total volume of 15 cc. with water and then adjusted to an approximate pH of 4.7.† Its reducing power is estimated by rapid titration with Tillman's reagent,‡ the endpoint being a reddish violet tinge persisting for at least 15 seconds.

The standard daily diet served these children was one considered high in vitamin C, containing 55 to 65 mg. per 24 hours. The coöperation of the nursing staff and the children was such that we have no hesitancy in stating that the full amount was taken daily. As controls, were 25 normal children on the same approximate diet.

Results. Of the 39 cases examined it will be noted that, with the exception of Cases 11 and 32,§ the highest excretion was 8.5 mg. The lowest was 2.8 mg. and the average of the 37 cases was 5.7 mg. This was distinctly below the control group of 25 normal children, the highest being 40.2 mg., the lowest 22.5 mg., and the average 29.2 mg.

Discussion. The low excretory level of 5.7 mg. in the tuberculous children falls definitely below 10 to 15 mg., the figure generally accepted as the minimum normal level. On the other hand, the control group average of 29.2 mg. was definitely above the latter figure, indicating that the diet was more than adequate for a normal child. This finding agrees with that of other investigators, Van Eekelen[9] for example, considers 50 to 60 mg. adequate for a body weight of 70 kg.

Such a low level of excretion in the tuberculous children would seem to point to increased destruction of the vitamin in the body. The exact mechanism, if such a condition exists, is not known. It must be remembered that vitamin C is thought to have its physiologic effect by a process of alternate reduction and oxidation, the reaction being apparently reversible. However, it is only in the reduced form that the vitamin can be assayed. This is done by measuring its reducing power against various oxidizing solutions. How then can we state that decreased urinary excretion of the vitamin in the reduced state indicates hypovitaminosis? This can be best answered by correlating clinical and laboratory data. Typical examples of gross vitamin C deficiency such as scurvy are found associated with greatly lowered excretion of the reduced vitamin in the urine. The administration of the vitamin in sufficient quantities

* Described by T. S. Bumbalo in thesis for M.S. (medicine), to be published.

† Two to three drops of 1 to 1000 Congo-red are placed in the depressions of a color plate. Ten per cent acetic acid is then added to the diluted urine in amounts of 0.10 cc. until a drop of the sample turns the indicator a purplish blue.

‡ Tillman's reagent, 20 mg. of 2:6 dichlorophenolindophenol is dissolved in 70 to 80 cc. of warm H_2O—cooled and made up to 100 cc. in a volumetric flask. One cc. is equivalent to 0.092 mg. of vitamin C as determined by our standardization. The solution, if kept in a dark bottle at 5 to 10° C., will not deteriorate appreciably for 7 days.

§ Cases 11 and 32 were receiving additional vitamin C from fresh fruit brought in by their visitors.

cures the patient clinically. At the same time the urinary output of the reduced vitamin is elevated to normal limits. This increased elimination suggests "saturation" of the tissues, the excess being spilled over into the urine. Such observations seem to justify a linkage of decreased urinary output of the vitamin in the reduced form with hypovitaminotic states.

Our findings in all 37 of the tuberculous group points definitely to hypovitaminosis, especially when one considers that the same diet in normal children resulted in comparatively high excretory levels. As previously mentioned, similar hypovitaminotic states are encountered in other acute and chronic infections. The cause for such deficiencies, on a vitamin C adequate diet, is not altogether understood. Such terms as "increased vitamin C metabolism" are used, but do not seem to afford a satisfactory explanation. It is possible that hypovitaminosis C may exist in many cases of active tuberculosis in both children and adults, due to inadequacy of diet. As seen in our series the large daily intake of 55 to 65 mg. was definitely insufficient. At the present time we are conducting studies to determine the approximate number of milligrams necessary for the establishment and maintenance of saturation in these children.

TABLE 1.—URINARY VITAMIN C OUTPUT IN TUBERCULOSIS.

Case number.	Case.	Age.	Color, sex.	Type of tuberculosis.	Nutrition.	Urine output 24 hours (cc.).	Vitamin C output 24 hours (mg.).
1	L. B.	13	W. F.	Pulmonary, with cavity	Good	2000	8.4
2	J. B.	9	C. M.	Childhood, pleurisy with effusion	Fair	850	4.5
3	U. F.	7	C. M.	Pulmonary, with cavity	Fair	700	4.7
4	F. E.	13	C. M.	Pulmonary, with cavity	Poor	1000	2.8
5	T. C.	14	W. F.	Pulmonary, with peritonitis	Good	600	4.8
6	K. C.	13	W. F.	Adenitis	Good	900	8.1
7	R. M.	14	W. F.	Pulmonary, with cavity	Poor	1000	4.2
8	R. M.	14	W. M.	Pulmonary, with cavity	Poor	750	4.0
9	R. R.	11	W. M.	Spine	Fair	1050	8.4
10	J. Y.	11	W. M.	Spine and pulmonary	Fair	950	3.2
11*	J. C.	10	C. M.	Childhood	Poor	600	20.1
12	B. G.	9	W. F.	Childhood	Fair	900	7.2
13	L. B.	13	W. F.	Pulmonary, with cavity	Fair	940	3.2
14	S. M.	8	W. M.	Left hip	Poor	1100	8.4
15	B. F.	5	W. F.	Pulmonary	Good	650	6.5
16	M. B.	6	W. F.	Pulmonary	Fair	500	5.8
17	T. K.	11	W. M.	Spine and right knee	Poor	750	5.5
18	R. M.	4	C. M.	Pulmonary	Poor	400	3.9
19	W. M.	11	C. M.	Pulmonary	Poor	1000	4.5
20	D. C.	12	C. M.	Right knee	Good	750	7.5
21	C. D.	10	W. M.	Left knee	Poor	625	5.6
22	H. M.	11	C. M.	Childhood	Fair	1250	5.3
23	W. F.	10	C. M.	Pulmonary	Fair	850	4.2
24	J. J.	8	C. M.	Left knee	Good	520	8.3
25	H. B.	12	C. M.	Pulmonary	Good	850	4.5
26	F. T.	9	W. M.	Right knee	Fair	1150	6.8
27	R. B.	14	W. F.	Pulmonary	Fair	1000	7.3
28	J. N.	12	W. M.	Childhood	Good	1200	7.5
29	J. Y.	13	W. M.	Spine	Poor	1000	3.2
30	E. H.	13	C. F.	Childhood	Fair	550	6.7
31	W. M.	9	W. M.	Pulmonary	Poor	750	8.2
32*	D. N.	9	W. F.	Childhood	Fair	1050	45.0
33	C. P.	10	W. M.	Pleurisy with effusion	Poor	1100	5.8
34	B. O.	13	W. F.	Left hip	Good	800	6.5
35	C. G.	3	C. F.	Miliary	Poor	350	2.7
36	E. E.	7	C. F.	Childhood	Good	675	6.8
37	R. M.	10	W. F.	Childhood	Fair	1750	5.0
38	C. J.	5	C. M.	Childhood	Good	575	6.0
39	A. B.	13	W. M.	Pulmonary, with cavity	Fair	1250	3.8
							Average 5.7 mg.

* Received additional vitamin C from fresh fruit brought in by their visitors

Summary and Conclusions. 1. All 37 cases in the children's wards of the Tuberculosis Service of the Buffalo City Hospital showed a decreased urinary excretion of vitamin C (reducing power as determined by the dichlorophenolindophenol method) on a daily ration of 55 to 65 mg. daily. Their average was 5.7 mg. as compared with a 29.2 mg. average of the 25 normal control children. In general, the greater the severity of the tuberculous process, the lower the excretory figures.

2. All 37 cases must be classified as subclinical hypovitaminosis C. The administration of the vitamin in amounts sufficient to produce saturation, and consequently a normal urinary output, is indicated.

3. The usual type of diet is inadequate for tubercular patients. Supplementary addition of the vitamin would seem advisable.

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FURTHER NOTE ON CARCINOMA OF THE BREAST IN ONE OF HOMOLOGOUS TWIN SISTERS.

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As the twin referred to in my article in this *Journal* (**190**, 331, 1935) had at that time only been observed by me for 2 years without developing carcinoma of the breast, like her twin sister, it seems desirable to add a further note at this time. It can now be said that over a period of 8 years since the appearance of carcinoma in the twin no evidence of carcinoma in the sister has become manifest. The possibility that the twins are not really homologous cannot be completely disproved, in fact would not necessarily be disproved if an apparently single placenta had been found. However, the fact that both persons are of the same sex, and of the same general appearance and constitution, would make it at least probable that they are homologous.

BOOK REVIEWS AND NOTICES.

PEDIATRIC DIETETICS. By N. THOMAS SAXL, M.D., F.A.C.P., F.A.A.P., Associate and Lecturer in Diseases in Children, New York Post-Graduate Medical School, Columbia University; Assistant Attending Physician, Babies' Ward, New York Post-Graduate Hospital, etc. Foreword by ADOLPH G. DESANCTIS, M.D., F.A.A.P., Director of Pediatrics at the New York Post-Graduate Medical School and Hospital, Columbia University. Pp. 565; 57 illustrations and 2 colored plates. Philadelphia: Lea & Febiger, 1937. Price, $7.00.

THIS book is filled, perhaps overfilled, with data and advice concerning the composition, classification and metabolism of children's foods, infantile digestion, maternal nursing, artificial infant feeding, diets, recipes, and menus. Its largest part is organized into a series of chapters on the dietetic management of the important pediatric diseases, a number of chapters being contributed by collaborating specialists. A thick appendix contains height-weight tables, physiologic data, well-selected bibliographies and long tables on the calorie, vitamin and mineral content of foods. The style is readable, the typography good and the index comprehensive.

The presentations are sound, conservative, and usually good, expressing in the main standard pediatric practices. Fads, fancies and freak diets are at a minimum, although the reader might prefer less emphasis on the proprietary infant-feeding preparations. One would like to see also some discussion of soft curd, vitamin D fortified, and other market milks, lead poisoning, raw banana feeding, flash pasteurization, and especially the splendid work of the American Medical Association's Council on Foods. All in all, however, the book should prove useful to the practitioner who desires a one-volume treatise on practical pediatric dietetics.

I. W.

CLINICAL ENDOCRINOLOGY. By SAMUEL A. LOEWENBURG, M.D., F.A.C.P., Clinical Professor of Medicine, Jefferson Medical College, Philadelphia; Assistant Visiting Physician, Philadelphia General and Northern Liberties Hospitals and Eagleville Sanatorium for Consumptives, etc. Foreword by HOBART A. REIMANN, M.D., Professor of Medicine and Clinical Medicine, Jefferson Medical College, Philadelphia. Pp. 825; 184 illustrations, 37 charts and tables. Philadelphia: F. A. Davis Company, 1937. Price, $8.00.

IN this book the author has followed the conventional outline of a medical text. There is an introductory chapter which stresses history taking and physical examination in the endocrinopathies. There follow chapters on the pineal, pituitary (anatomy, physiology and pathology), diseases of the pituitary thyroid, parathyroid, pancreas, adrenals, female and male gonads, undetermined endocrine activities (properly brief) and a final chapter on the significance of laboratory findings. Each chapter follows, for the gland considered, the outline, history, anatomy, physiology, pathology, diagnosis and treatment. Remarks on endocrine interrelationships are included in several chapters. This outlins the scope of the book, and its purpose, namely, the clear presentation of the diseases of the ductless glands.

This purpose has been fulfilled by the concise and fluent writing and the orderly arrangement of the material. An underlying regard for general

medicine is evident throughout, especially in the sections on differential diagnosis. The inclusion of tables of body weights and measurements, laboratory data in normals, etc., will save time for the reader. The sections on treatment are to be highly commended. Fads are avoided, a large number of alleged remedies are discarded on the grounds of experience and the judgment of a sound and practical clinician is apparent. The book is supplied with good illustrations.

In such a large subject omissions or minor errors will occur and each reader will note these for himself. The Reviewer failed to find any mention of Crooke's work on the hyaline degeneration of the basophilic cells of the pituitary—a work of clinical pathologic coördination which should appeal to all clinicians. Perirenal air injection for the Roentgen ray diagnosis of adrenal tumors, and the use of low sodium diets in the diagnosis of Addison's disease were not mentioned.

The glucose-tolerance test of 0.8 gm. per pound (=1.75 gms. per kg.) is described. This should be per pound of *ideal* weight in fairness to the functioning tissues of the obese or edematous. Perhaps the English system of a standard dose of 50 to 100 gms. is as satisfactory as any for routine use. The bitterling test is described (correctly) as a measure of estrogenic substances, but since it also appears to measure male sex hormones and perhaps other sterols, its insertion in a text might be postponed.

The last comment merely indicates the problem of deciding when an up-to-date fact is established, and no work on endocrinology can escape this difficulty. The author has avoided controversial writing and clinically his conclusions are correct. In adopting this worthy impartiality, however, there is a certain lack of stimulating or advanced ideas. Thus the possible rôle of the pituitary in thyroid disease is referred to but not discussed with the emphasis which even conservative authorities are giving this subject today.

These comments do not prevent the Reviewer from agreeing with Dr. Reimann's statement in the Foreword that the author's experience in teaching, as presented in this book, will be of real value to students and practitioners.

F. L.

A Text Book of Medicine. By American Authors. Edited by Russell L. Cecil, A.B., M.D., Sc.D., Professor of Clinical Medicine, Cornell University Medical College; Associate Attending Physician, New York Hospital, New York City; Associate Editor for Diseases of the Nervous System; and Foster Kennedy, M.D., F.R.S.E., Professor of Neurology, Cornell University Medical College; Director, Department of Neurology, Bellevue Hospital, New York City. Pp. 1614; 42 illustrations. Fourth edition, revised and entirely reset. Philadelphia: W. B. Saunders Company, 1937. Price, $9.00.

The popularity of this well-known text is demonstrated by the fact that the fourth edition appears only ten years after the first. The editor states that he has tried to bring every subject up to date and thereby give the student an authoritative presentation of present-day internal medicine. Several "new" topics—at least they were not included in previous editions—have been introduced. A retiring age for contributors has been established so that the present edition contains an unusually large number of new treatises on old subjects.

In the preface to the first edition, the editor stated that the rapid growth of medical science during the last few years has made it almost impossible for a single individual to master the entire field. He has therefore divided the field among 130 contributors. Most of these contributors are recognized authorities on the subjects they discuss. Consequently the percentage of erroneous statements, including the propagation of traditional misbeliefs

from older texts is at an unusually low level. Many of the treatises are excellent although some others are dull and difficult to read. From this point of view there are all gradations from good to bad.

On the whole, this is a very useful textbook of medicine and can be heartily recommended to students. The Reviewer feels that every student whose ambitions soar beyond the mere passing of examinations should equip himself with at least two textbooks of medicine. This book should be one of the two. C. W.

A Textbook of the Practice of Medicine. By Various Authors. Edited by Frederick W. Price, M.D., C.M., F.R.C.P., F.R.S. (Edin.), Consulting Physician to the Royal Northern Hospital; Senior Physician to the National Hospital for Diseases of the Heart, London, etc. Pp. 2038; 112 illustrations. Fifth edition. New York: Oxford University Press, 1937. Price, $12.50.

Five editions and a total of 14 printings since 1922 are ample proof of the popularity of this excellent British text. The work of 28 contributors, it shows the uniformly high standard of excellence of its various sections which such multiple authorship can achieve more easily than can a single author. At the same time, good editorial supervision has taken care of covering all topics, with proper emphasis and without duplication. The work differs from American texts in its inclusion of sections on tropical diseases, psychiatry and dermatology, the latter of somewhat questionable value in the absence of illustrations. The index is exceptionally good.

R. K.

Personality and the Cultural Pattern. By James S. Plant, M.D., Director, Essex County Juvenile Clinic. Pp. 432. New York: The Commonwealth Fund, 1937. Price, $2.50.

In this thought-provoking book the author presents the method of attack on personality problems which he has developed and used for 15 years in the several institutions of Essex County. In addition, he advances his philosophy of mental hygiene—or prophylactic psychiatry—which is somewhat unique since it emphasizes the dynamic effect of environment and the whole social order upon the individual personality—especially in childhood.

In fair and logical exposition he analyzes the approaches offered by the mental hygiene methods customarily used, formal psychiatric methods employed in clinics, and psychoanalysis. Except in obscure cases of mental disease in more advanced age groups, he believes that the latter two procedures are either too time-consuming and too highly formalized or even entirely unfited for the early variations of personality which occur so frequently in childhood. The author prefers to build up a system of mental hygiene based on recognition and study of the "casual breakdown." To accomplish this adequately he suggests that such cases be handled in informal clinics attached to courts' schools, factories and churches. He visualizes the highly trained psychiatrists as organizers and teachers rather than as workers in the units. He believes that in this way the relations of society, environment and the individual personality, together with their effects on each other are studied and integrated better.

This whole presentation together with the author's observations on our whole social order should prove to be of great interest to physician, sociologists, ministers, educators and officials in our crimnal and juvenile courts. The author's methods of attack in the hands of those who have had the opportunity to observe them in his clinics should prove to be a contribution to the study of childhood behavior and to the development of sound personality patterns. E. T., Jr.

A BRIEF RULE TO GUIDE THE COMMON-PEOPLE OF NEW-ENGLAND. How to Order Themselves and Theirs in the Small Pocks, or Measels. [First published in 1677–78, reprinted in 1702 and 1721–22.] By THOMAS THACHER. Facsimile reproductions of the three known editions with an Introductory Note by HENRY R. VIETS, M.D. Pp. 54; illustrated. Price, $1.50.

A DISCOURSE UPON THE INSTITUTION OF MEDICAL SCHOOLS IN AMERICA. [Reprinted from the first edition, Philadelphia, 1765.] By JOHN MORGAN. With an Introduction by ABRAHAM FLEXNER. Pp. 63; 1 illustration. Price, $2.00.

ADAPTATION IN PATHOLOGICAL PROCESSES. [Reprinted from Transactions of the Congress of American Physicians and Surgeons, 1897, vol. IV, pp. 284–310.] By WILLIAM H. WELCH, M.D., LL.D. With an Introduction by DR. SIMON FLEXNER. Pp. 58; 1 illustration. Price, $1.50.

The above 3 books (publications of the Institute of the History of Medicine, The Johns Hopkins University, Fourth Series, Bibliotheca Medica Americana, vols. I, II and III, respectively.) Baltimore: The Johns Hopkins Press, 1937.

AMONG the several contributions that our only American Institute of the History of Medicine makes to medical history are its five series of publications: 1, Monographs; 2, Texts and Documents; 3, Noguchi Lectures; 4, "Bibliotheca Medica Americana;" and 5, the *Bulletin* of the Institute. The 3 volumes listed above are the first 3 of the fourth series, reprints of important medical Americana, with introductory historical, biographic and bibliographic sections. These sections may occupy but a few pages, as in the Morgan reprint; or far surpass the original text in size, as in the case of Thacher's Brief Rule. In the case of the Brief Rule, in fact, the original text does not even appear listed in the Table of Contents, as its 3 editions occupy 16 pages of illustrations only. E. K.

AN OUTLINE OF GENERAL PHYSIOLOGY. By L. V. HEILBRUNN, Associate Professor of Zoölogy in the University of Pennsylvania. Pp. 603; 122 illustrations. Philadelphia: W. B. Saunders Company, 1937. Price, $5.00.

THE nature of the subject discussed in this book is very clearly outlined in the introductory chapters. General physiology is defined as the science which deals with all forms of living material. The general physiologist believes that all life has something in common; his search is often for the most common denominator of living processes; his aim is to discover, so far as possible, the nature and mechanism of living matter. Professor Heilbrunn points out that modern progress in biology has resulted from the fortunate realization that living things, no matter how diverse they appear in externals, are fundamentally much alike; and it is quite certain that much of the hope of the future lies in the possibility of using simpler types of protoplasm in order to discover the general characteristics of living things.

The early chapters of the book deal briefly with the structural, physical and chemical properties of living systems. Then follows a more detailed survey of the activities of living things: general metabolism, nutrition, digestion, respiration, secretion, excretion. In further chapters are then taken up the energy output due to these activities, the production of mechanical energy, of heat, electricity and light. The environment and the effect of environment is next considered, and in connection with this the adaptability to changes in the environment. This logically leads to a discussion of the reaction to various stimuli, one of the most important of the properties of living system. The book concluded with chapters on reproduction and on senescence.

Throughout the book the author has directed attention to the original literature, to the sources of information. The interested student or research worker will find this bibliography very helpful, and judiciously selected.

Professor Heilbrunn's book can sincerely be recommended to the physician and the biologist who would gain a broad survey of the manifestations of life, and the undergraduate as a well written presentation of a subject which is, in truth, the foundation of all the biologic sciences.

B. L.

DIE STERNALPUNKTION ALS DIAGNOSTISCHE METHODE. By PROF. DR. HANS SCHULTEN, Hamburg-Eppendorf Pp. 82; 2 illustrations by Gisela Krämer; 16 plates in color. Leipzig: Georg Thieme, 1937. Price, RM. 18.

THIS little monograph, dealing with biopsy of the sternal bone marrow as a diagnostic procedure, is the best of its kind that has appeared thus far. It is notable especially because of the excellence of color plates which reproduce faithfully the appearance of blood cells and their progenitors as seen in normal and various disease states. The author discusses technical methods, cytologic features of the several developmental series and a practical scheme of hemopoiesis. Over half of the test is devoted to a special section in which bone marrow findings (his own and those of others) in disease of the blood-forming organs are concisely described. Six pages of references furnish a fairly comprehensive bibliography.

The puncture and aspiration method of obtaining marrow was employed (which the Reviewer regards as inferior to the trephine method), but the author is careful to point out possible fallacies and reasons for discrepancies in differential counts.

American medical publishers can well take note of such paper-backed volumes as a relatively inexpensive but quite adequate medium.

R. C.

CLINICAL ALLERGY. By LOUIS TUFT, M.D., Chief of Clinic of Allergy and Applied Immunology, Temple University Hospital; Associate in Immunology, Temple University School of Medicine; Director of Laboratories, Pennsylvania Department of Health, Philadelphia. Introduction by JOHN A. KOLMER, M.D., DR. P.H., D.Sc., LL.D., L.H.D., Professor of Medicine, Temple University; Director of Research, Institute of Cutaneous Medicine, Philadelphia. Pp. 711; 82 illustrations. Philadelphia: W. B. Saunders Company, 1937. Price, $8.00.

THE past 25 years have seen an amazing increase in our knowledge of the field of human hypersensitiveness. This information is found in a rapidly growing literature, much of it speculative and controversial. Some major and detailed texts have been written primarily for those who make this field their chief interest. A number of manuals have also been published for the guidance of patients. With the exception of Vaughn's book (and even this was primarily written with the patient in view) nothing has appeared for the benefit of the student and general practitioner. The current medical texts, even the systems, are woefully deficient in this regard. Dr. Tuft's book, written for the practitioner and student, therefore fills a real need. In fact, it overfills it, for it has grown to proportions probably beyond those originally intended by the author. This, however, is the practitioner's, if not the student's, gain. The subject matter is exceedingly well arranged and well presented. The first section considers the fundamental principles of allergy and anaphylaxis, including diagnosis and treatment. The second section discusses clinical allergy from the standpoint of etiologic agents (*e. g.*, allergy to serum, to drugs, foods, pollens, bacteria and physical agents). The third section takes up the clinical

manifestations of allergy, notably asthma, hay fever, gastro-intestinal allergy and migraine. The allergic skin conditions are described in the fourth section, together with chapters on allergy in children and in relation to certain other medical specialties. Of particular value and interest is the appendix with its descriptions of methods, lists of allergens, diets and recipes. There is a considerable bibliography, conveniently arranged by subjects, which especially the young allergist will find helpful. The book is heartily recommended. R. K.

BIOLOGICAL TIME. By P. LECOMTE DU NOÜY, Chief of the Division of Molecular Biophysics, Pasteur Institute, Paris; formerly Associate Member of the Rockefeller Institute. With a Foreword by ALEXIS CARREL, M.D., Rockefeller Institute for Medical Research. Pp. 180; 31 illustrations. New York: The Macmillan Company, 1937. Price, $2.00.

DURING the World War the author studied with Carrel the rate of healing of wounds, work which was published and obtained world-wide recognition. In the present volume the author takes the reader "behind the scenes" of this investigation, informally discusses the origin of the problem in the hospitals of France, how difficulties arose and were met. He goes through the successive steps in mathematical analysis and shows how equations were fitted to the data. It was found that a man of 40 takes nearly twice as long, for example, to cicatrize a wound as a man of 20; a man of 50 four times longer than a child of 10. A similar effect of age was reported by Carrel, who found that fibroblasts in tissue culture grow much faster in the serum of a young chicken than in that of an old one.

From these two instances the author makes the generalization that the tempo of life is very rapid in childhood and progressively declines with age (though the author concedes that not all biologic phenomena are slowed up by age in the same proportion as cellular activity). From these considerations he develops the conception of "physiological time" as opposed to sideral time. The year seems much longer to the child than to the adult. Everything occurs "as if sideral time flowed four times faster for a man of fifty than for a child of ten." Therefore "when we take physiological time as a unit of comparison physical time no longer flows uniformly."

The Reviewer is not qualified to criticize the author's philosophic discussion of time, and possibly for this reason, found the earlier part of the book dealing with the author's experiments much more interesting.

M. McC.

VERHANDLUNGEN DER DEUTSCHEN GESELLSCHAFT FÜR KREISLAUFFORSCHUNG. X. Tagung zu Bad Nauheim vom 13.–14. März, 1937. Hauptthema: Kreislauf und Innere Sekretion. Herausgegeben von PROF. DR. EB. KOCH, Bad Nauheim. Pp. 320; 140 illustrations. Dresden: Verlag von Theodor Steinkopff, 1937. Price, Rm. 15.

OF the 35 contributions to this session, 16 bear on the main theme—the circulation and internal secretion. Beginning with an address by Sir Henry Dale on vasodepressor substances, such topics were presented as Adrenalin as a Circulatory Hormone (H. Rein-Göttingen); the Hypophysis and the Circulation (F. Schellong); Hormones and Circulation in Relation to Pregnancy (W. Haupt); the Circulation in Disturbances of Blood Sugar Regulation (M. Bürger); the Circulation in Disturbances of the Thyroid (G. W. Parade) and so on. One can safely say that the usual high standard of these Proceedings is maintained in the present volume. E. K.

DIE HOMÖOPATHISCHE BEHANDLUNG DER AUGENKRANKHEITEN. By DR. KARL ERHARD WEISS, Augenarzt in Stuttgart. Pp. 182. 2 Durchgesehene und Verbesserte Auflage. Stuttgart: Hippokrates-Verlag G.M.B.H., 1937. Price, paper, Rm. 6.80; Bound, Rm. 8.00.

THE first part of this book is an alphabetical list of homeopathic remedies, which may be used in the treatment of eye diseases. Most of these have long since disappeared from the pharmacopœia. The second part deals with various diseases of the eye and suggests the use of the remedies mentioned in the first half. It is rather interesting to find a book advocating the use of remedies whose pharmacodynamic action has been demonstrated to be *nil*, such as gelsemium, oleander, pulsatilla, and so on.

F. A.

ALLERGY. ITS PRACTICAL APPLICATION. Expressly Prepared for Physicians and Students of Medicine, Containing Practical Points Necessary for the Care of Patients with Asthma, Hay Fever, Urticaria, Eczema, and Other Allergic Conditions. By J. A. RUDOLPH, M.D., Associate Clinician in Charge to the Department of Allergy, Mt. Sinai Hospital; Consultant in Allergy, Cleveland Y. M. C. A. Pp. 224. Philadelphia: Dorrance & Co., Inc., 1937. Price, $3.00.

EXPRESSLY prepared for students and practitioners, the book to a large extent achieves this aim, in that it contains much practical information for the care of patients with allergic conditions. There is, however, much valuable space given to material not essential to the primary purpose of the book, for instance, the preparation of extracts, and unnecessarily detailed presentation of theories, often controversial. Errors and omissions are commonest in first editions, but some should not even occur in "firsts," such as the use of the word "intradermal," some of the prescription Latin, the statement that 90% of hay fever cases are of the fall variety, the failure to mention the use of adrenalin by atomizer.

R. K.

THE HUMAN MIND. By KARL A. MENNINGER, M.D. Pp. 517; 17 illustrations. Second edition, corrected, enlarged and rewritten. New York: Alfred A. Knopf, 1937. Price, $5.00.

THE chapters of this popular volume discuss principles, personalities, symptoms, motives, treatments and applications. Personalities include those who tend to show difficulties when subjected to an additional strain; some, however, may lead successful lives: 1. Organic disease type—crippled personalities such as those redeemed from tuberculosis. 2. Hypophrenic type—stupid personalities. 3. Isolation type—lonely personalities which are of two sorts: "constitutionally" unsocial subjects who prefer to be left out of things, and the "wistful derelicts who long to dive into the stream." 4. Schizoid type—queer personalities, some of whom succeed because they are queer while others fail by reason of this. 5. Cycloid type—moody personalities. 6. Neurotic type—that large group of frustrated personalities that may become successful through struggle and compromise. 7. Antisocial type—perverse personalities that may show intelligence but are lacking in emotional and volitional functioning. The chapter on motives is chiefly concerned with psychoanalysis. There are minor errors and omissions: Peter the Great in one place is an epileptic and in another, a paranoiac; according to authorities he was neither, but was a pronounced *tiqueur*. Neglect to mention family care of mental patients which has definite advantages in selected cases. The author gives a non-technical, dramatic and at times delightful presentation of mental mechanisms that will appeal to lay readers and students.

N. Y.

THE ROENTGENOLOGIST IN COURT. By SAMUEL WRIGHT DONALDSON, A.B., M.D., F.A.C.R., St. Joseph's Mercy Hospital, Ann Arbor, Mich. Pp. 230. Springfield, Ill.: Charles C Thomas, 1937. Price, $4.00.

THIS book contains a wealth of sound advice concerning medical testimony. While the author primarily concerns himself with radiology and the law, it contains much of value to all members of the medical profession. With their medico-legal work on the increase, radiologists should find increasing use for this volume. It not only defines the radiologist's status as an expert witness, but cites numerous cases of importance in guiding radiologic testimony.

The chapter, "Doctor, Take the Stand," is well worthwhile reading by any physician who contemplates going into court as a witness for the first time.

P. H.

TUMORS OF THE NERVOUS SYSTEM. An Investigation of the Most Recent Advances. The Proceedings of the Association, New York, December 27 and 28, 1935. (Association for Research in Nervous and Mental Disease, Vol. XVI.) Editorial Board: EDWIN G. ZABRISKIE, ANGUS M. FRANTZ, CLARENCE C. HARE. Pp. 493; 213 illustrations and 64 tables. Baltimore: The Williams & Wilkins Company, 1937. Price, $7.50.

HERE are included tissue culture of gliomata, by R. G. Canti, J. O. W. Bland and Dorothy S. Russell; metabolism of brain, spinal cord and meningeal tissue, by S. Bernard Wortis; metabolism of brain tissue by Joseph Victor and Abner Wolf; antigenic properties of brain tumors, by Arthur Weil and Erich Liebert; effects of irradiation on gliomas, by C. H. Frazier and Bernard J. Alpers; gliomas of the central nervous system, by Arthur Elvidge, Wilder Penfield and William Cone; ependymomas, by James W. Kernohan and Eleanor M. Fletcher-Kernohan; meningiomas, by Joseph H. Globus; tumors and cysts of the cerebellopontine angle, by Leo Alexander; cerebrospinal fluid in brain tumor, by Frank Fremont-Smith; localization of brain tumors by tests of olfactory function, by Charles A. Elsberg; pneumencephalographic diagnosis of corpus callosum tumors, by Cornelius G. Dyke and Leo M. Davidoff; clinico-pathologic study of astrocytomas, by R. W. Waggoner and Konstantin Lowenberg; long postoperative survivals in cases of intracranial tumor, by Louise Eisenhardt; tumors of peripheral nerves, by Arthur Purdy Stout, George F. Laidlaw and Cushman D. Haagensen; tumors of peripheral nerve system, by Frank E. Adair and Jay McLean.

Appreciative discussion followed exhibition of tissue cultures of gliomata, with cinematograph demonstration of cells cultivated *in vitro;* however, the method was not offered as supplanting the histologic diagnostic procedure. Olfactory tests in localization of tumors brought out an interesting discussion; the author holds that smell is primarily physical, rather than chemical. The research association is to be congratulated upon having arranged a scientific program that elicited such an enlightening discussion.

N. Y.

EARLY MEDIEVAL MEDICINE. With Special Reference to France and Chartres. By LOREN C. MACKINNEY, PH.D., Professor of Medieval History, University of North Carolina. (Third Series, Vol. III. Publications of the Institute of the History of Medicine, The Johns Hopkins University. The Hideyo Noguchi Lectures.) Pp. 247; illustrated. Baltimore: The Johns Hopkins Press, 1937. Price, $2.75.

FEW indeed are those students and writers who are thoroughly trained in both medicine and history; and as professional historians have for the most part concerned themselves very little with medical matters, we find

that most essays in medical history emanate from the physician and all too often show the defects of the amateur. Though the author may be correct that it is harder to learn medicine than to learn history, still we are inclined to believe that the trained historian will do a better job with most medical subjects than *vice versa*. Certainly the little volume under consideration would support this view. Tracing in three lectures the progress of medicine through: 1, early medieval times, 2, Merovingian and Carolingian France, and 3, at Chartres in the 10th and 11th centuries, the author confirms the modern view that the Dark Ages, especially in their latter half, were far from pitch black in medicine as well as in general culture. In general, he steers a middle course, rejecting the claims both for a Carolingian medical renaissance and for a sharp decline of medicine in the next 2 centuries. As to Chartres, one has to be already a zealous supporter of that glorious cathedral to become enthusiastic about its Gerbert and its Filbert and the slender contributions of the cathedral school. The subject has been carefully prepared—the 284 textual notes occupy a good quarter of the book—and should now benefit many more than those who were fortunate enough to hear the third Noguchi lecture series. E. K.

EYESTRAIN AND CONVURGENCE. By N. A. STUTTERHEIM, M.D. (RAND.), ARTS (STAATS-EXAMEN., HOLLAND), Part-tim Ophthalmic Surgeon to the Johannesburg School Clinic, Transvaal Education Department; late Assistant, Eye Clinic, University, Leyden. Pp. 89; 2 illustrations. London, H. K. Lewis & Co., Ltd., 1937. Price, 7s. 6d.

THIS book, of interest only to ophthalmologists, is a thesis on deficiency of convergence as a cause of eyestrain. The author's overenthusiasm for his point of view is best illustrated by the following quotation on page 45: "I have not yet met with a case of eyestrain where there was not a marked degree of asthenovergence." The treatment suggested is orthoptic exercises, which build up the convergence power. F. A.

THE MICROMOTIST'S VADE-MECUM (BOLLES LEE). A Handbook of the Methods of Animal and Plant Microscopic Anatomy. Edited by J. BRONTË GATENBY, M.A., PH.D. (DUBL.), B.A., B.SC., D.PHIL. (OXON.), D.SC. (LOND.), Professor of Zoölogy and Comparative Anatomy, Trinity College, Dublin; and THEOPHILUS S. PAINTER, A.B. (ROANOKE), A.M., PH.D. (YALE), Professor of Zoölogy, Texas University, U. S. A., with 10 COLLABORATORS. Pp. 784; 11 illustrations. Philadelphia: P. Blakiston's Son & Co., Inc., 1937. Price, $9.00.

A LARGE section on botanical microtomy technique has been added to this edition, in an effort to place on the shelves of botanists and zoölogists a book that will give each a chance to learn some of the methods of the other. New chapters have also been added on frozen sectioning and vital staining; many have been revised or rewritten. While it continues to be an excellent reference book on unusual methods, it is not as up-to-date on routine measures as might be hoped for. G. S.

THE THERAPEUTIC PROBLEM IN BOWEL OBSTRUCTIONS. A Physiological and Clinical Consideration. By OWEN H. WANGENSTEEN, B.A., M.D., PH.D., Professor of Surgery, University of Minnesota, and Surgeon-in-Chief, University of Minnesota Hospital. Pp. 360; 90 illustrations. Springfield, Ill.: Charles C Thomas, 1937. Price, $6.00.

PART I of this monograph, awarded the quinquennial Samuel D. Gross Prize in 1936 by the Philadelphia Academy of Surgery, considers the therapeutic problems of bowel obstruction, including the effects of distention, the recognition of bowel obstruction, the treatment of bowel obstruc-

tion, with a concluding chapter for summary and conclusions. In Part II are discussed the diagnosis, the picture presented by patients with bowel obstruction, guiding principles in treatment, choice of operation, operation and postoperative treatment. Part III deals with specific types of clinical obstruction.

It is little short of amazing that the author, whose fundamental researches in this field make him the best qualified American surgeon to discuss the subject, could have covered so much material in such a brief space. The important literature is briefly but adequately reviewed. All subject matter is clearly and concisely presented. The illustrations are excellent. No clinical surgeon can afford to fail to place this volume in his library; it fills a need and will long serve as a model for surgical monographs. I. R.

A Monograph on Veins. By Kenneth J. Franklin, D.M., M.R.C.P., Tutor and Lecturer in Physiology, Oriel College; University Demonstrator of Pharmacology; Assistant Director of the Nuffield Institute for Medical Research, Oxford. Pp. 410; 46 illustrations. Springfield, Ill.: Charles C Thomas, 1937. Price, $6.00.

In the eager search for knowledge leading to control of arterial disease, it is not surprising that the veins, of less clinical importance, should have been comparatively neglected. Nevertheless, one is astonished to learn that this is the first English monograph on the subject and impelled to congratulate author and publisher on remedying the deficiency. The author, at the instigation of Professor Gunn, now Director of the new Nuffield Institute for Medical Research, has for several years been doing research work on the venous system. He now has integrated this with existing knowledge on the subject in a review primarily intended for others engaged in such studies, but useful of course, for the profession at large. Medical history, embryology (by Keith Richardson), anatomy—human and comparative—experimental physiology, pharmacology, and the clinic have all been drawn upon to give a complete picture of these structures and "the important parts they play in the harmonious interactions within the living organism." E. K.

NEW BOOKS.

Erforschung und Praxis der Wärmebehandlung in der Medizin. Einschliesslich Diathermie und Kurzwellentherapie. 2. Frankfurter Konferenz für medizinisch-naturwissenschaftliche Zusammenarbeit am 13. und 14. Mai, 1937. Mit Unterstützung der Stadt Frankfurt a. M. Herausgegeben von Dr. B. Rajewsky, o. Professor für physikal, Grundlagen der Medizin, Frankfurt a. M., und Dr. H. Lampert, o. Professor für physikal, Therapie, Balneologie und Klimatologie, Frankfurt a. M. Pp. 185; 82 illustrations. Dresden: Theodor Steinkopff, 1937. Price, Paper, Rm. 8.; Bound, Rm. 9.50.

The Immunological Reactions of the Filterable Viruses. By F. M. Burnet, E. V. Keogh, and Dora Lush. (From the Walter and Eliza Hall Institute, Melbourne.) (Reprinted, with original page numbers, from "The Australian Journal of Experimental Biology and Medical Science," Vol. XV, Supplement to Part 3.) Pp. 136; 27 illustrations. Obtainable from The Librarian, University of Adelaide, Adelaide, South Australia, and H. K. Lewis Co., Ltd., London. Price, 10/–.

An Analysis of the De Generatione Animalium of William Harvey. By Arthur William Meyer, Professor of Anatomy, Stanford University. Pp. 167; illustrated. Stanford University, Calif.: Stanford University Press, 1936. Price, $3.00.

Short Years. The Life and Letters of John Bruce MacCallum, M.D., 1876–1906. By ARCHIBALD MALLOCH. Pp. 343; illustrated. Chicago: Normandie House, 1938. Price, $3.50.

The Rôle of Chemiotaxis in Bone Growth. By A. P. BERTWISTLE, M.B., CH.B., F.R.C.S. (EDIN.). Pp. 59; 32 illustrations. London: Henry Kimpton, 1937. Price, 8/6.

Protoformotherapy in Treatment and Prevention. Fifteen Years of Research on New Scientific Bases of Therapeutics. By DR. N. E. ISCHLONDSKY, Paris. Extended edition of three successive lectures delivered before the Egyptian Medical Association at the University of Cairo in March, 1936, under the title: "The Internal Secretion of the Embryonic Tissues, its Biological Significance and its Importance in Practical Medicine." Pp. 237; 68 illustrations on 40 plates. London: Henry Kimpton, 1937. Price, 21/–.

A Dissertation on Acute Pericarditis. By OLIVER W. HOLMES. Introduction by JAMES F. BALLARD, Director, Boston Medical Library. Pp. 39. Boston: The Welch Bibliophilic Society, 1937. Price, $7.50.

The British Encyclopædia of Medical Practice. Including Medicine, Surgery, Obstetrics, Gynecology and Other Special Subjects. Vol. 5, Endoscopy of Respiratory Tract to Goitre. Under the general editorship of SIR HUMPHRY ROLLESTON, BT., G.C.V.O., K.C.B., M.D., D.Sc., D.C.L., LL.D., Emeritus Regius Professor of Physic, Cambridge; sometime President of the Royal College of Physicians of London. With the assistance in a consultative capacity of F. R. FRASER, M.D., F.R.C.P., G. GREY TURNER, D.CH., M.S., F.R.C.S., JAMES YOUNG, D.S.O., M.D., F.R.C.S. (EDIN.), F.C.O.G., SIR LEONARD ROGERS, K.C.S.I., M.D., LL.D., F.R.C.P., F.R.C.S., F.R.S., F. M. R. WALSHE, O.B.E., M.D., D.Sc., F.R.C.P. Pp. 683; 139 illustrations. London: Butterworth & Co. (Publishers), Ltd. Price, $12.00.

Love and Happiness. Intimate Problems of the Modern Woman. By I. M. HOTEP, M.D., with a Prefatory Note by DR. LOGAN CLENDENING. Pp. 242. New York: Alfred A. Knopf, 1938. Price, $2.00.

Fever Therapy. Abstracts and Discussions of Papers Presented at the First International Conference on Fever Therapy, College of Physicians and Surgeons, Columbia University, New York City, March 29, 30, 31, 1937. Edited by 9 Members of the American Committee. DR. WALTER M. SIMPSON, Dayton, Ohio, Chairman; DR. WILLIAM BIERMAN, New York City, Secretary. Pp. 483. New York: Paul B. Hoeber, Inc., 1937. Price, $5.00.

Neuland in der Heilkunde. By DR. HENRI HIRSCH. Pp. 87. Basel: S. Karger, 1937. Price, Fr. 3.20.

The new land lies in the recognition that health and disease are a function of the acid-base-equilibrium. This is "the base of all harmonious processes of the human organism," and its disturbance is "the base of all diseases." It is also the base of psychic processes. The pamphlet cannot be regarded as scientific. W. E.

Die Diffusionsanalyse am Blutplasmagel. Ein Neuer Weg der Blutforschung. By RUDOLF BUCHER. Pp. 123; 70 illustrations (30 in color). Basel: Benno Schwabe & Co., 1937. Price, Fr. 30.

Tissue Reactions in Bone and Dentine. A morpho-biological Study of the Formation and the Dissolving of Bone and Dentine. By ÅKE WILTON, M.D., Assistant Professor and Lecturer in Pathology at the Caroline Institute, Stockholm. Pp. 194; 64 illustrations and 5 plates in color. London: Henry Kimpton, 1937. Price, 15/–.

Physiologie de l'écriture Cursive. By DR. H. CALLEWAERT. Pp. 123; 57 illustrations. Bruxelles: L'edition Universelle, S.A., n.d. (No price given.)

The Physician's Business. Practical and Economic Aspects of Medicine. By GEORGE D. WOLF, M.D., Attending Otolaryngologist, Sydenham Hospital; Attending Laryngologist, Riverside Hospital, New York City. Foreword by HAROLD RYPINS, A.B., M.D., F.A.C.P. Pp. 384; 57 illustrations. Philadelphia: The J. B. Lippincott Company, 1938. Price, $5.00.

Man Against Himself. By KARL A. MENNINGER. Pp. 485. New York: Harcourt, Brace & Co., 1938. Price, $3.75.

The Negro's Struggle for Survival. A Study of Human Ecology. By S. J. HOLMES, Professor of Zoölogy in the University of California. Pp. 296; 10 illustrations and 50 tables, also Appendix of 31 tables. Berkeley, Calif.: University of California Press, 1937. Price, $3.00.

Digestive Tract Pain. Diagnosis and Treatment, Experimental Observations. By CHESTER M. JONES, M.D., Assistant Professor of Medicine, Harvard University; Physician, Massachusetts General Hospital. Pp. 152; 5 illustrations. New York: The Macmillan Company, 1938. Price, $2.50.

Surgical Pathology of the Diseases of the Neck. By ARTHUR E. HERTZLER, M.D., Surgeon to the Agnes Hertzler Memorial Hospital, Halstead, Kansas; Professor of Surgery, University of Kansas. Pp. 237; 206 illustrations. Philadelphia: J. B. Lippincott Company, 1937.

"To the Surgeon the neck is the most interesting region of the whole body. . . . . A presumptive diagnosis is possible in the clinic and by the time the operation is finished very few lesions should be obscure. These few are found almost wholly in the affections of the lymph glands. Here also the pathologist finds his greatest difficulties. . . . . The present tendency to treat all lesions of the neck by irradiation without a definite pathologic diagnosis is to be deplored. What surgeons have learned is in grave danger of being lost by this practice. It is still the practice in my clinic to remove lesions by block dissection, thus insuring a positive diagnosis. This is then followed by irradiation if and when needed. Only by such a practice is it possible to know what has been cured, should permanent relief be obtained." (From the Author's Preface.)

Theoretical Principles of Roentgen Therapy. Edited by ERNST A. POHLE, M.D., PH.D., F.A.C.R., Professor of Radiology; Chairman, Department of Radiology and Physical Therapy, University of Wisconsin, Madison, Wisconsin. Foreword by W. EDWARD CHAMBERLAIN, B.S., M.D., F.A.C.R., Professor of Radiology in the Temple University School of Medicine, Philadelphia. Pp. 271; 132 illustrations. Philadelphia: Lea & Febiger, 1938. Price, $4.50.

NEW EDITIONS.

Macleod's Physiology in Modern Medicine. Edited by PHILIP BARD, Professor of Physiology, Johns Hopkins University School of Medicine, with 8 Collaborators. Pp. 1051; 355 illustrations and 103 tables. Eighth edition. St. Louis: The C. V. Mosby Company, 1938. Price, $8.50.

Approved Laboratory Technic. Clinical, Pathological, Bacteriological, Mycological, Parasitological, Serological, Biochemical and Histological. By JOHN A. KOLMER, M.D., DR.P.H., SC.D., LL.D., L.H.D., F.A.C.P., Professor of Medicine, Temple University; Director of the Research Institute of Cutaneous Medicine, Philadelphia, etc., and FRED BOERNER, V.M.D., Assistant Professor of Bacteriology, School of Medicine and Graduate School of Medicine, University of Pennsylvania; Bacteriologist, Graduate Hospital, Philadelphia. Pp. 893; 380 illustrations and 12 colored plates. Second edition, rewritten, revised and reset. New York: D. Appleton-Century Company, Inc., 1938. Price, $8.00.

PROGRESS
OF
MEDICAL SCIENCE

PATHOLOGY AND BACTERIOLOGY

UNDER THE CHARGE OF
W. L. HOLMAN, M.D., C.M.,
PROFESSOR OF BACTERIOLOGY, UNIVERSITY OF TORONTO, TORONTO, CANADA,
AND
G. L. DUFF, M.A., M.D., PH.D.,
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SULPHANILAMIDE AND SIMILAR COMPOUNDS IN CHEMOTHERAPY.

THE chemical compound, which started a most exciting series of researches in the field of chemotherapy, was synthesized by Mietzsch and Klarer[45] in 1932 and patented on Christmas Day of that year.[79b] It was the addition of the sulphonamide group to the molecule of the azo dye chrysoidine which resulted in producing the 2:4 diaminoazobenzene, 4′-sulphonamide, called for brevity's sake Sulphonamide chrysoidine, and known in the medical literature under the trade names "Streptozon" and later "Prontosil."

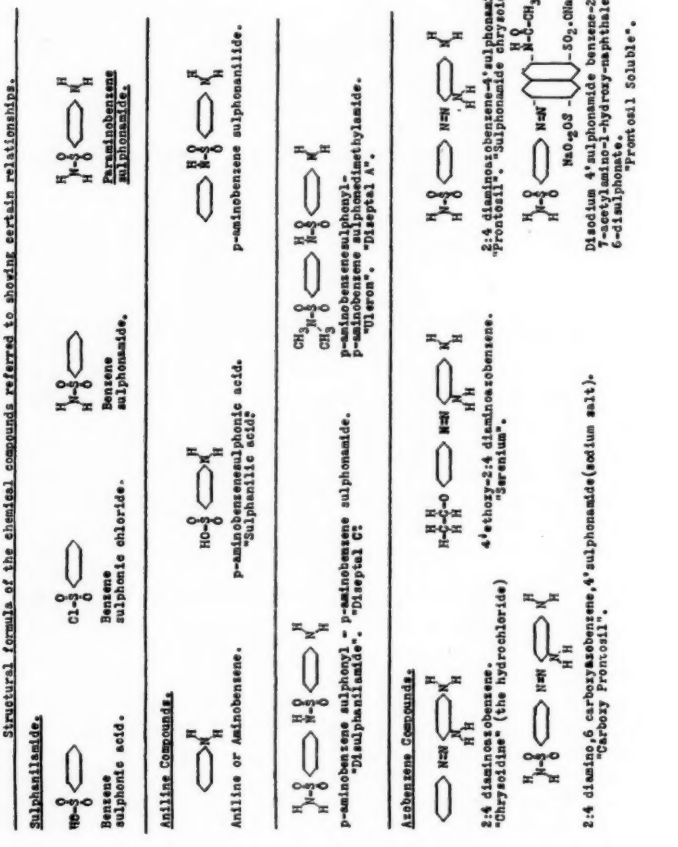
This compound was lifted out of the turmoil, caused by the appearance year after year of chemotherapeutic agents heavy with claims of antibacterial efficiency, when Domagk[45a] discovered a new principle. This consisted in the experimental observation that the *in vitro* antiseptic qualities of the azo dyes was reduced by the addition of the sulphonamide group; but, and this was the heart of the discovery, the therapeutic action *in vivo* was not only not reduced but when it was given to mice after intraperitoneal injections of fatal doses of hemolytic streptococci the mice did not die. The fine coöperation of the bacteriologists and the chemists led to the production of "Prontosil" and "Prontosil Soluble," and in the hands of clinicians there followed a series of dramatic curative results which recalled the early days of dioxydiamidoarsenobenzol—the "Ehrlich-Hata 606" or Salvarsan.

The earliest reports on the use of "Streptozon" were those of clinicians, and these appeared before the basic experimental work in Domagk's laboratory had been published. Of course, the compound had not been given out for trial in human infections without the necessary laboratory evidence, which had been obtained the same year as the synthesis of the drug, although this was not available to the general medical world until February, 1935.[45a] It is only of historic interest

that Foerster[94d] in May, 1933, reported favorable results in a boy with widespread staphylococcus infection, that Grütz[94d] in 1934 had had good results in the treatment of different types of erythema, and that Veil[125] in the same year had noted a decrease in the sedimentation rate of the red cells and benefit in rheumatism when these cases were treated with "Streptozon."

Table I.

Structural formula of the chemical compounds referred to showing certain relationships.



There was little or no interest aroused by these early reports; but in the early months of 1935 the tide began suddenly to rise, and by December, 1937, the writer of this review is all but overwhelmed by the flood of articles from clinicians, laboratory workers and chemists in all parts of the world, so that he must of necessity take note only of what he believes are the most important currents to which his attention has been drawn.

Chemistry. The chemical substances which are being developed in this field are referred to as chrysoidine, aniline, or more recently sulph-

anilamide derivatives. However, the compounds being tested are no longer confined to these derivatives since the stimulus of the experimental technical procedures, and the highly favorable results obtained in clinical cases has broadened the scope of this world-wide research. The structural formulas shown in the table are designed to indicate

Table I (ctd.)

Compounds with additions to the amino radical of sulphanilamide.

p-anisobenzenesulphonamide-formaldehyde sulphoxylate.	p-aminobenzenesulphonamide-formaldehyde bisulphite of sodium.	p-benzylamino-benzenesulphonamide. "Septazine."	p-(γ phenyl-propyl-amino) benzene sulphonamide-4(γ. disulphonate of sodium. "Soluseptazine."	
Sulphone Compounds (O=S=O or a compound of it with carbon):				
4:4 diaminodiphenyl-sulphone.	4:4 dinitrodiphenyl-sulphone.	4:4 diaminobenzene-sulphonanilide tartrate.	4:3 diaminobenzene-sulphonanilide.	
Sulphide Compounds:				
4:4 diaminodibenzenesulphide. "Thioaniline."	2:2 pyridylsulphide dihydrobromide.			
Pyridine Azo Compounds:			**Sulphoxide:***	
2,hetoxypyridyl 5, 5, azo, 2, 6 diaminopyridine. "Neotropin."	Benzene azo-2,6 diaminopyridine. "Pyridium."		4 nitro-4'aminodiphenyl sulphoxide.	
Other Compounds:				
p-benzenesulphonamide-pyridine- -carbonamide-4-carboxylate of sodium.	aminonaphthalene-4-sulphonate of sodium.			

* Reported as a potent antiendotoxic agent (Gonococcus and B. aertrycke) Levaditi and Vaisman, (Presse méd. 45, 1371, 1937: Compt. rend. Acad. d. sc. 205, 1106, 1937).

certain relationships among a number of substances which have already been reported and they may be helpful in following the present-day lines of research.

Domagk,[45] Hörlein[79] and others have outlined the historic steps leading to the discovery of "Prontosil." The comparative failure of

chemotherapy in bacterial infections had been outstanding, and the theory suggested was that the higher differentiation of protozoa and spirochetes rendered them more subject to chemical attack, in that certain functions essential to the life of these higher organisms could be affected with relative ease by a number of chemicals. A method suitable for testing such chemicals was the first necessity before progress could be made, and it must be recognized that success followed the trial and error system, and then only after a very long series of compounds had been tested. Here and there, more or less favorable results in experiments on animals narrowed the field until "Prontosil" was discovered with a low toxicity for animal tissue, a negative bactericidal effect *in vitro*, but a high therapeutic activity in artificially infected animals.

The publication of the chemical formula of "Prontosil," showing it to be a sulphonamide chrysoidine compound, naturally stimulated the search to discover what part of the molecule was chiefly responsible for the striking results. Many hundreds of substances have been studied by German, French, English and American investigators, and at the time of writing no fully satisfactory explanation has been reached, particularly because other compounds of different chemical structure have been found which give essentially the same type of results. Many of these have not had sufficient clinical application to permit evaluating them at present, but it should be emphasized that only the threshold of bacterial chemotherapy has been passed.

The first important step toward the chemical clarification was made by Tréfouël *et al.*,[155] when they found after testing a long series of substances that all those which gave favorable therapeutic results contained the para aminobenzenesulphonamide group, and that this compound by itself was as effective as the diaminoazobenzenesulphonamide compound. Further study by Fourneau *et al.*,[50] Buttle *et al.*,[30] Goissedet *et al.*,[62] Fuller,[56] Long and Bliss[94a] and many others, established the correctness of this discovery. At the suggestion of Fuller the name sulphanilamide, later adopted by the Council on Pharmacy of the American Medical Association,[1] has passed into current use and has largely, but unfortunately not completely, supplanted a long list of proprietary and other names among which those most commonly used have been "Prontosil Album" and "Prontolyn."

Sulphanilamide, as can be seen in Table 1, is derived from benzene sulphonic acid and its relationship to aniline and sulphanilic acid is also there indicated. The importance of the molecular structure has been shown by the authors referred to above. The para position of both the amine and the sulphonamide groups is essential for its effect in experimental infection. Buttle *et al.*[30] believed the amine attachment to the benzene ring was more important than the sulphonamide group alone, having found that the anilide derivative of sulphanilic acid was as active as the amide.

It is generally accepted that the azobenzene compounds, which are effective in experimental and clinical application, such as the sulphonamidechrysoidine ("Prontosil"), the carboxy modification (Carboxy-Prontosil) of Girard,[60] and the naphthalene compound ("Prontosil Soluble") owe at least the major part of their potency to the sulphanilamide group, which is set free on reduction of these compounds in the body. There is much evidence that this occurs both by finding the

sulphanilamide in the urine and experimentally by reduction with a number of substances such as sodium formaldehyde "sulfoxalate,"[94a] stannous chloride[56] and sodium hydrosulphite,[11, 56] and cysteine hydrochloride.[19b] It has also been suggested[94a] that the streptococcus may cause reduction and a number have indicated that in the presence of inflammation such reduction may occur. Similarly, with the formaldehyde sulphoxylate (Bauer[128]) and the formaldehyde bisulphite[62] compounds, as well as with the more widely known "Septazine" and "Soluseptazine," the sulphanilamide group would appear to be readily released on reduction. The disulphanilamide ("Disulon"), Bauer,[128] (named Diseptal C or D.B.32)[79b] was a little more effective on streptococci given subcutaneously and less than one-fifth as acutely toxic as sulphanilamide in the hands of Rosenthal.[128] Hörlein[79b] said it was superior to sulphanilamide against staphylococcus as well as against gas gangrene in mice, and it has been used in treating human gonorrhea. The closely related dimethyl compound with the trade name "Uleron" (Diseptal A or D.B.90)[79b] has also been clinically tested in gonorrheal infections, as has a similar compound to the above but with a single CH_3 radical called Diseptal B (D.B.87). None of these three compounds, according to Hörlein,[79b] can be reduced to sulphanilamide.

All the substances containing the sulphanilamide radical do not act alike. They differ in toxicity, solubility, rapidity of excretion and in their effects on different bacteria. These differences are explained in a number of ways. The ease and rate of reduction of the more complex compounds may vary, so that the sulphanilamide released may be in a nascent or more potent state for longer periods in some than in others. The method of administration will alter the conditions favoring reduction, as will the presence of inflammation and other metabolic changes. The mere fact that the species of animal changes the relative efficiency of these drugs indicates the intricate factors which may be involved.

The second molecule set free when the azo or other linkage is broken must be considered in the final result produced by these compounds, and undoubtedly may alter the effects in the body. It has been recognized by a number of investigators[60a] that the effects of the different compounds do not run parallel with their sulphanilamide content. A mere glance at Table I will show the variety of secondary molecules which may be formed. Some of these may be changed or rendered inert in the body, but a great deal more work will be needed before the facts are fully known.

The large amount of evidence confirming the view that sulphanilamide is a remarkably efficient therapeutic agent against a variety of infectons makes it evident that equally extensive tests must be made with the growing number of other compounds which have given comparable results in experimental infections in animals. There are the sulphone compounds (diaminodiphenyl and the dinitrodiphenyl)[32] and the two diaminobenzenesulphonanilides,[161] the sulphide compounds, thioaniline[60b] and the pyridylsulphide,[88] the para benzenesulphonamide pyridine compound,[81] and the aminonaphthalene sulphonate.[30]* These various preparations do not contain the sulphanilamide radical. In the case of the first two sulphone compounds the conversion to sulphanilamide is highly improbable,[32] but these are effective in curing strep-

* In recent studies Levaditi and Vaisman used sulphoxide compounds (see Table I).

tococcal infections in mice—the diaminosulphone in doses one hundred times less, and the dinitrosulphone in doses the same as sulphanilamide, the first being twenty-five times as toxic and the second less toxic for mice but not for rabbits.[32, 60b] The two anilide compounds[161] have a polyvalent action and have a low toxicity for mice. Gley and Girard's report[60b] on thioaniline gave further evidence that several compounds, in which the sulphur has different chemical functions, are able to arrest the evolution of streptococcal infection in mice. The pyridylsulphide[88] has a definite curative effect on intradermal streptococcal lesions in rabbits but it is more toxic than "Prontosil Solution." The pyridine compound,[81] although it has the benzene sulphonamide group, has a pyridine nucleus replacing the amine group. It is probably not converted into sulphanilamide in the body. It has a powerful antistreptococcus effect *in vivo*, is readily soluble and is practically free from toxicity for mice and rabbits.[81] The naphthalene compound of Buttle *et al.*[30] has a therapeutic index about the same as sulphanilic acid, and both of these are less active than sulphanilamide. I have included in the table a number of compounds referred to by Domagk,[45] "Serenium" to show its relationship to chrysoidine and Prontosil, and the pyridine azo compounds "Neotropin" and "Pyridium" since these have been used to combat infections particularly of the urinary tract.

It would seem, even from this abbreviated review, that the substances therapeutically active against experimental infections with different bacteria are relatively numerous. Of these, sulphanilamide has been studied most completely and the importance of its molecular structure is evident. Sulphur in various functional states as sulphonamide, sulphonate, sulphone or sulphide* appears as a common constituent of this group of active substances and it is found as part of the radicals in many of the derivative compounds. The amine radical in the para position of the benzene ring seems to have some determining effect along with the sulphur containing radicals. Although the benzene ring is the basic constituent of almost all the effective compounds, Kolmer[88] believed the pyridyl nucleus offers greater possibilities for future study, particularly when it contains divalent sulphur in the molecule.

The solubility in water of these various preparations differs widely. Sulphanilamide is soluble 0.4 to 0.8% as the base (Hawking[74] gave 1.5 gm. in 100 ml. of water at 20° C.) and as the hydrochloride it is freely soluble in water; but this latter solution is acid and causes necrosis on subcutaneous injection.[30] It is of interest that Tréfouël,[155a] Fourneau[50] and Goissedet[62] used the hydrochloride. Buttle[30] found it too acid for use, Tréfouël (1937)[155b] used the amine, the sodium and the chloride preparations but found the latter too acid for toxicity tests, and Barlow[10] also reported that both the sodium and the hydrochloride were too irritant. Foulis and Barr[49] reported one patient in whom the drug (the base?) was ineffective until dilute hydrochloric acid was given preceding its ingestion, and Paton and Eaton[120] referred to 2 patients who were given hydrochloric acid to aid absorption, and they considered that these patients may therefore have received a greater effective dose. With very few exceptions it may be presumed that the base is the form in which sulphanilamide has been administered, but it may be converted into a more irritating form in the body. The sulphonanilide derivative is of low solubility. Disulphanilamide is also poorly soluble. Rosen-

* In recent studies Levaditi and Vaisman used sulphoxide compounds (see Table I).

thal[128] found it less effective against streptococci than sulphanilamide when given by mouth but it was much less toxic than the latter. "Uleron" is also only slightly soluble in water but is readily soluble in dilute alkalies.

"Prontosil" is effective both as the base and as the hydrochloride,[45c] the latter is used in a 0.25% solution, but the base is often used in the tablets because the hydrochloride has a more bitter taste and is not well tolerated orally. To increase its absorption from the stomach hydrochloric acid is also given.[22, 29, 45a, 111] Jaeger[83] believed the base had a greater affinity for the skin than the chloride and also that it increased the clotting of blood on local application. It would appear that it is the hydrochloride which is usually referred to under the name "Prontosil."

"Prontosil Soluble" is, as the name implies, much more easily soluble, being used in 2.5 to 4% solutions and it has largely replaced the original "Prontosil." Both "Prontosils" have been frequently administered together, one by mouth, the other intramuscularly or intravenously, so that clinical statistics are confused as to which may be the better therapeutic product.

The formaldehyde sulphoxylate derivative is water-soluble, "Septazine" is only slightly soluble in water and is prepared in tablet form. The "Soluseptazine" is used in a 6% solution corresponding to a 2% solution of sulphanilamide. The sulphone compounds (diamino and dinitro)[32] are poorly soluble, the first 0.01%, the second is insoluble.

Evidence for the Curative Action. The therapeutic efficiency of these drugs has been established on the basis of animal experiments, chiefly mice. Intraperitoneal injections of mouse-virulent beta hemolytic streptococci has been the procedure in the great majority of the laboratory studies, and this is followed (occasionally preceded) by the particular drug which is given as a rule subcutaneously or *per os*. The evidence demanded was the death of the untreated controls within 24 to 48 hours and the prolonged survival or a marked delay in death of the treated animals.

Domagk[45a] using "Prontosil" indicated clearly that after the injection of about 10 fatal doses of the streptococcus (0.3 cc. of a 1:100,000 dilution of a 24-hour culture usually killed in 24 hours) the drug in doses one-tenth to one-fiftieth of the tolerated amount when it was given over a period of 3 to 5 days saved the life of a large majority of the mice. The most convincing evidence for its activity was obtained by a comparison of smears from the peritoneal exudate of the treated and untreated mice, the streptococci quickly disappearing from the former while in the latter they multiplied rapidly and caused a septicemic death. Backed by the clinical reports on a number of serious human streptococcal infections in which striking curative results had been obtained by the drug, this experimental work was repeated in France,[92] England,[36] and America.[94] It was soon discovered that a necessary condition for successful results in mice was the use of strains of hemolytic streptococci of a rather surprisingly high virulence.[36a, 92, 94, 116] Hemolytic streptococci from human infections are rarely of high virulence for mice, and usually a long series of passages was needed before satisfactory virulence was obtained. Further interesting observations were that it was rare to obtain a prolonged survival of all the animals treated however the

drug was given; that living cocci could often be obtained from the blood or tissues of treated mice long after the untreated controls had died; and that those animals dying after long periods from streptococcal infections quite often failed to show the pathologic lesions characteristic of streptococcal deaths. Levaditi[92c] had mice dying of streptococcus septicemia 18 to 19 days after apparent cure, and Long and Bliss[94] had such delayed deaths after 39 days and even after 63 days and 128 days. The explanation of such results is particularly difficult if the drug is to be considered in the ordinary sense as a germicidal or even as an antiseptic agent.

The criteria of efficiency suggested by Buttle[30] were: (1) the number of lethal doses against which the drug will protect when given under optimal conditions; (2) the latest time after the infection when the drug is still able to save the animal. The minimal protective dose has also been used, and the prophylactic value of the drugs when given at various times preceding the infection. The latter seems to depend largely upon the rate of elimination of the drug from the body which depends more or less upon its solubility. It is indeed difficult to properly assess and compare the various drugs which are being used in these experiments, and if this is true under the comparatively rigid conditions of laboratory study how much more difficult it will be in the clinical evaluation. Whitby[161] gave a scheme for reporting these experimental results, under which the lethal dose must remain constant in both size and virulence. To obtain the average days of survival the number of mice surviving each day is multiplied by the number of days and the sum of these is divided by the number of mice used. Any particular drug in its action against any bacterium can thus be compared under the above conditions.

Beta hemolytic streptococci have been used in the majority of these experiments because their virulence can be kept reasonably constant, and the intraperitoneal route is the most convenient for obtaining comparable results. However, various sites have been used in these studies as will be seen later in this review. Berger[15] particularly employed the subcutaneous route to produce streptococcal abscesses in mice and gave "Prontosil Soluble" by mouth 5 hours later and repeatedly up to 5 days. He was thus able to prevent the general spread of the infection and the establishment of fatal metastases in a high percentage of his mice. He wondered whether all streptococci will be found to be as susceptible as the strain he used. It was at first believed that the drug "Prontosil" acted in a rather specific manner against streptococci as is indicated in the trade name "Streptozon," although the early clinical reports gave evidence that it was effective in a wider field,[94d] and Domagk[45a] himself had found it more or less active against staphylococci. These views were largely the result of the technical procedure in the experimental work and were soon radically modified. The beta hemolytic streptococcus has been shown to be particularly susceptible to all these drugs, and a number of workers considered that the streptococci of the Lancefield Group A, which includes all the primary and serious hemolytic streptococcal infections in man were almost exclusively susceptible. Long and Bliss[94c] found the A and B groups were affected, but in 4 patients with D group infections the drugs were valueless. It is true that most of the laboratory work and

the dramatic clinical results in the human have been obtained with strains of this group. There has not been enough comparative work done, however, to make this suggestion tenable. Levaditi[92c] had good results using a hemolytic strain (M) from a horse with glanders. Tréfouël[155b] used a highly virulent animal strain (Pion) in his experiments on rabbits. Davesne and Brunschwick[41] believed they controlled a streptococcic epizoötic among newborn lambs by the use of "Prontosil" tablets, Stevens[149] in a letter reported the apparent cure of bovine mastitis by means of sulphanilamide, and Seastone,[143] using a strain of group "C" which gave an acutely fatal infection in animals, was able to prevent death with sulphanilamide. On the other hand, Colebrook and his colleagues,[36, 38] Long and Bliss[94] and others had no convincing evidence that the drugs were effective in man for other than Group A infections. It is clear that the evidence on either side is comparatively meagre, and until more detailed experiments are available it would seem wise to withhold final judgment. The character of the infection induced and the relative virulence of the organisms seem to be the important factors.

Other technical methods have also been used with hemolytic streptococci. Domagk[45a] treated rabbits successfully with experimental chronic infections in which the joint tissues were swollen and endocarditis was present. Tréfouël[155b] found the rabbit no better than the mouse for testing a series of chemical compounds. They used it particularly in studying the prophylactic or protective effect of sulphanilamide. Bürgers[29] carried out extensive experiments on 112 rabbits, 85 guinea-pigs and about 500 mice using intravenous, subcutaneous and intraperitoneal infections. Gay and Clark[58] showed sulphanilamide to be effective in experimental empyema in the rabbit. Kolmer[88] used skin and joint infections in rabbits in a study of eight compounds including the pyridyl sulphide mentioned above. Campbell[33] quoted Long as believing that "the rabbit is not a particularly good animal, because of metabolic peculiarities, on which to test sulfanilamide." The reason for this belief is not clear to the Reviewer, but until a fuller knowledge is available as to the mode of action of these drugs every factor must be seriously considered, particularly before applying the experimental results in animals to the explanation of the curative effects in man.

There is practically universal agreement that infections with alpha hemolytic streptococci (*Str. viridans*) are unaffected by these compounds; but even here the character of the lesion in subacute endocarditis, which is the infection usually considered, practically precludes the successful action of any agent, and I have not seen any report dealing with animal experiments using a highly virulent strain of *Str. viridans*.

Staphylococcus infections in rabbits were included in Domagk's original article.[45a] He had favorable results, particularly in acute spreading infections, but even in chronic infections curative effects were obtained although not as regularly as with streptococcus infections. It is of passing interest that the first article in the medical literature on the use of these chemicals reported a marked chemotherapeutic effect in a case of a generalized staphylococcal infection.[94d] The results in human infections will be discussed later. The difficulty in studying staphylococcus experimentally has been to find a strain of sufficient

and constant virulence so that the untreated controls died with some regularity. It seemed to involve the same problem as was noted for streptococci with low virulence. Buttle[31] used a bovine strain and was successful in protecting mice by sulphanilamide against a thousand fatal doses. Hörlein[79b] reported that the three "Diseptal" compounds were strikingly more effective against staphylococcus in mice than sulphanilamide. Helmholz[76] found the urine of sulphanilamide treated patients to be bactericidal against staphylococcus.

In the case of the pneumococcus group the results have been most variable. Domagk[45] reported that "Prontosil" had some effect on Type III pneumococcus but not on Types I and II, and in 1936[45c] that sulphanilamide was more effective against pneumococci than was "Prontosil." Nitti[117] reported a direct inhibitory action of sulphanilamide *in vitro*. Buttle[32] found the diaminosulphone compound to be twenty times more effective than sulphanilamide in prolonging life in the mouse, but complete cure was uncertain. The explanation offered was the prolonged action of this drug due to its low solubility which is considered necessary for protection against pneumococcus in mice. Rosenthal (1934)[127a] showed that formaldehyde sulphoxylate was effective in curing mice infected with one particular strain of Type I pneumococcus but gave negative results with all other types and strains. In a later study[127b] he found sulphanilamide possessed a certain degree of chemotherapeutic activity against 7 strains of Types I, II and III infections in mice. He[127a, 129c] also found it bactericidal and bacteriostatic for pneumococcus *in vitro*, although no such effect was obtained with streptococci and other organisms. He and his associates[128] further showed that this drug was much more effective against pneumococci intraperitoneally in rats than in mice and rabbits. Branham and Rosenthal[23] had much better results when sulphanilamide was combined with specific serum therapy. Cooper *et al.*[40] using subcutaneous injections of about 10 lethal doses of a highly virulent Type III pneumococcus in the mouse was able to demonstrate a definite curative effect with sulphanilamide. They point out that because of the extreme susceptibility of the mouse to pneumococcus these results are of greater significance than the lethal doses would suggest. They[66a] later obtained good results with the same drug in rats infected intrabronchially with the same strain of organism suspended in mucin, and by this same technique further showed[66b] that Type I pneumococcus was controlled as effectively by the drug as by the specific serum. A combination of drug and serum gave somewhat better results. On the basis of these results they recommend its trial in man and include the suggestion that the occasional complicating hemolytic streptococcus infection would also be controlled. A more detailed study[39a] on some 64 rats infected as above with the strain of Type III pneumococcus showed that the effectiveness of the drug treatment varied inversely with the interval between infection and initial treatment, and with the size of the infecting dose. The treatment would appear to enable the surviving rats to overcome the associated toxemia which usually proved fatal in the controls, since the course and extent of the pneumonia was not appreciably affected. In a fifth report[39b] they used 2 strains of Type II pneumococcus and here also found the drug at least as effective as the specific antiserum, the combined drug and antiserum giving no better results. Kreidler[90] used rabbits infected with a Type I pneumococcus

of high rabbit virulence intracutaneously and found that sulphanilamide if given early and in sufficient dose eliminated the pneumococcus from the blood, reduced the fever, cured the local lesion and favored recovery in most of the treated animals. Schmidt[135] used a Type XIV pneumococcus in mice intraäbdominally and was able to save all the mice if treatment with the drug was started within 4 hours of the time of infection even when large doses of organisms were used. Whitby[161] gave rather higher efficiency figures for his two diamino anilide compounds than for sulphanilamide using a Type I pneumococcus in mice. All of these results are most encouraging for an effective chemotherapy in pneumonia.

In the experimental study of Proom with meningococcus—first mentioned by Buttle[30]—the bacteria in 5% mucin were injected intraperitoneally in mice. Proom[123] showed that the protection afforded by sulphanilamide against Types I and II meningococcus is somewhat greater than that against hemolytic streptococci when the drug is given at once, either subcutaneously or *per os*. It prevented septicemia and death, and he had some evidence that the drug reached the cerebrospinal fluid when given by mouth. Rosenthal[128] confirmed Proom's findings, using the same technique, and compared the curative effects of a number of related compounds given subcutaneously. The disulphanilamide gave better results than sulphanilamide, "Septazine" gave only a fair percentage of cures, and "Prontosil" and "Prontosil Soluble" were not as effective. The formaldehyde sulphoxylate compound and the sulphonanilide were found inferior to the others. A further study,[23] using 20 strains of meningococci (Types I, II and III), demonstrated that sulphanilamide even in single doses 2 hours after the infection, especially when given subcutaneously, caused the survival of a large percentage of mice. A comparison with serum therapy gave irregular results, but when these were combined the results were definitely better and demonstrated a synergistic action rather than an additive one. Levaditi and Vaisman[92e] tested both "Prontosil" and sulphanilamide and found these drugs curative in the experimental infection induced by meningococci (Types A, B and C) in mucin. There was about the same irregularity as they had had in their experiments with streptococci. Whitby[161] used Proom's technique, confirmed his findings and reported that his sulphonanilide tartrate was just as effective as sulphanilamide, the average day survival being 4.2 for the former and 4.1 for the latter. "Septazine" and "Soluseptazine" had no appreciable effect. Brown[27] compared the relative value of sulphanilamide and a potent antiserum and found that 8 mg. of the drug was practically equal to 0.1 cc. of the serum. They also confirmed Branham and Rosenthal[23] that a combined therapy was more effective, and further noted that the percentage of positive blood cultures taken 8 hours after the infection was lower than when either serum or drug was used alone.

Because of technical difficulties there has been practically no experimental work with these drugs on the gonococcus,* but they have been widely used in the clinic because they are excreted by the kidney, and the favorable results with meningococcus offered some hope that they might be effective. For much the same reason the basic laboratory study with many other bacteria is relatively meager. Buttle[31] did

* See recent work of Levaditi and Vaisman (note under Table I).

preliminary determinations for members of the Salmonella group and other Gram-negative bacilli, obtaining a 75% survival in mice infected with *B. typhosus* and treated with sulphanilamide, although positive cultures could be obtained from blood or tissues a week or even a month later. Helmholz[76] found the urine after sulphanilamide to be bactericidal for most bacteria including *Proteus*, *B. coli*, *B. aërogenes* and others. Bürgers[29] found "Prontosil Soluble" had no effect against virulent Friedländer bacillus in mice nor against 7 strains of *B. abortus* in guinea pigs. Despite these results Berger and Schnetz[16] reported a favorable response to this drug in a patient with *Brucella abortus* infection. Nitti[117] had negative results with *B. typhi murium* in mice treated with sulphanilamide and the evidence from the spontaneous deaths in treated mice caused by a paratyphoid organism as mentioned by Levaditi[92] and by Long[94] is against these drugs having any useful effect on these Salmonella types. Bliss and Long[19b] used *Clostridium welchii* by intraperitoneal injections in mice previously given sulphanilamide by the same route and found that the drug protected the mice. The 3 untreated controls died in less than 7 hours from toxemia. Hörlein[79b] quoted Domagk as having had better results with the three "Diseptal" drugs than with sulphanilamide against gas gangrene in mice.

Campbell[33] was unable to obtain any effect with sulphanilamide in experimental syphilis in the rabbit. Buttle[31] had negative results with *Trypanosoma equiperdum* in mice as well as with avian malaria in canaries. Diaz de León,[43] however, had successful results with "Prontosil" in 15 cases of benign tertian malaria in man.

In the few virus diseases studied, Levaditi and Vaisman[92] found "Prontosil" without effect in experimental herpes (encephalitic strain) and lymphogranuloma inguinale. Kelson[84] also had negative results in experimental poliomyelitis in the monkey. The only successful result reported to date is that of Rosenthal, Wooley and Bauer[129] with "Prontosil" against the virus of lymphocytic choriomeningitis in mice. These same authors had negative results with other related compounds and were unable to obtain any effect against the virus of encephalitis (St. Louis type) or the virus of influenza.

Clinical Results. The publications reporting the clinical application of many of these new drugs to all manner of human diseases are so numerous that only mere references can be given to most of these. To avoid needless repetition dosage, methods of administration and other details will not be considered despite the fact that these are often of great importance. It may be said, however, that the intravenous route was used in the early days of "Prontosil" and "Prontosil Soluble," but the oral and subcutaneous routes are almost exclusively used today, and that other methods of administration have been employed only under special conditions. Since the chemical methods have been developed by Fuller,[56] and particularly by Marshall and his colleagues[98, 100] for determining the percentage content of sulphanilamide in the blood, urine, cerebrospinal fluid and other parts of the body, the most effective dosage can be very accurately determined. The mechanism of the excretion by the kidney[100c] is now better known and the distribution in the tissues according to their water content has been established.[100d]

Human Hemolytic Streptococcal Infections. *Erysipelas.* The evident difficulty in evaluating the results of any treatment of this disease

is its frequent natural tendency to recover with or without treatment. It is, however, often impossible to predict how severe an attack may be in the early stages, and as prompt treatment is advisable evidence must depend largely on statistics. In the very young and in the older age groups erysipelas is apt to be more severe so that somewhat greater weight should be given to the results in such cases. In the following condensed summary only the briefest notes are possible.

"Prontosil" was used in the early cases reported in 1935 both as tablets and in the 0.25% solutions. Gmelin[61] treated 10 children and normal temperature resulted on the second to third day in 9, and in 1 on the fifth. Schreus[139] considered the drug a specific and had no resistant case in 18 months. Klee and Römer[86] had rapid recoveries even in the aged. Imhäuser[82] reported 4 cures. Scherber[133] in a long report compared the drug to "Omnadin" (a lipoid preparation containing the fermentation products of non-pathogenic bacteria acting as a non-specific cell stimulant), and showed a reduction in the average day of fever in favor of "Prontosil." Roth[130] had a good result in a necrotizing erysipelas of an entire leg as well as in 2 other severe cases. Lampert[91] reported rapid clinical results and in 2 cases, despite a septicemia with purulent metastases, had a prompt cure. In 1936, Kramer[89] reported 23 cases in which the fever disappeared in 2 to 3 days and the temperature and pulse became normal in an average of 4.3 days, under other forms of treatment this was 11.3 days. He had two failures, apparently due to too small amounts of the drug. Scheurer[134] had his most striking results in this disease and considered it a specific for the severe septic cases—other cases recover without treatment. Valerio[156] reported success in 3 very severe cases in infants. Tonndorf[153] treated 22 with no failures, the patients became fever-free in 2 days and often in 1. If the temperature did not fall promptly he looked for a complication, believing this of value in differential diagnosis. Meyer-Heine and Huguenin[110] treated 150 cases with only two failures. They were particularly impressed by their results in 8 infants. Bloch-Michel[20] used "Septazine" in 250 cases. In 170 cases of face erysipelas 9 died and 8 of these were over 70, but a very severe infection in a patient of 73 was cured. Frankl,[52] out of 40 patients treated, did not lose a case. He particularly stressed the disappearance under treatment of the immature white cells and considered a drop in the total white cell count a favorable prognostic sign. Meyer zu Hörste[111] gave statistics indicating the frequent high mortality from erysipelas in the newborn ranging from 27.3 to 80%. He used "Prontosil" base orally in his infant cases and accounted for at least one of his failures, if not two, by the failure of absorption due to lack of gastric acidity. When dilute hydrochloric acid was given with the base his patient promptly responded. Hartl[72] did not find "Prontosil" of any benefit in 21 cases, none of them septic, in that the cure was no more rapid than in the controls. Anghelescue *et al.*,[3] during 1934 and 1935, treated 631 cases and compared the results of ultra-violet light therapy in 20, antistreptococcus serum in 17 and "Prontosil" in 12. The average days in hospital in all was 15. Under light therapy this was reduced to 8, under serum to 12 to 14 and under "Prontosil" to 6. The responses to "Prontosil" therapy, as others have reported, were prompt. In 1937 they[4] reported particularly on the good results obtained in 6 cases

with severe kidney complications and in which "Prontosil" not only cured the erysipelas but also improved the conditions in the kidney. Becker,[13] in a comparison of 50 cases treated by the drug with 50 under other treatments, reported a reduction in the duration and always the arrest in the spread of the infection in the treated cases, particularly when the drug was given orally. Peters and Havard[121] had striking results in 47 cases treated by "Septazine." The youngest patient was 4 months old and 3 were aged 70, 81 and 87. The temperature returned to normal as a rule in 24 (in 31) or 48 hours (in 12), only 1 having pyrexia up to the fifth day, and the spread was arrested within 24 hours in all cases.

Breen and Taylor[24] studied 46 cases in 35 of which sulphanilamide was used. Regression occurred in 48 hours in all but 2, one of these, a baby, responded soon after, and the other was an adult who later developed a lung abscess. They were particularly impressed by their remarkable results in the age group 50 to 65. Whitby[161] successfully treated 2 cases of erysipelas and 1 of cellulitis with "Septazine." Snodgrass and Anderson,[146] in a very carefully controlled study of 312 cases, treated 104 by ultra-violet light, 106 by "Prontosil" tablets (10 "Prontosil Soluble"), by a combined treatment in 54 and with antistreptococcus toxin in 48. Fifteen died and are not considered in the tables but the percentage of deaths (4.8) is much lower than in other years in the Glasgow area. In those receiving "Prontosil" the death rate was 2.5%, for the others 6.6. The tabulated results cannot be abbreviated but it may be noted that spread stopped in 2 days in 98% in the drug-treated cases and in 75% of the others; pyrexia over 3 days in 8% of the former and in 41% of the latter; toxemia persisted after 3 days in 21% of the "Prontosil" cases and in 39% of the others. In a symposium on Sulphanilamide Therapy[150] are reports of 42 cases in infants and young children in which 37 responded, 3 died and 2 were still in hospital. Among these Basman and Perley[12] had 3 cases of abscess formation in their 9 patients and they speculate, on the basis of the immunity in erysipelas being largely cellular, that the drug may abort the infection and on its withdrawal the bacteria still present may lead to abscess formation.

Despite the obvious difficulties in reaching a definite conclusion the evidence seems incontrovertible that this group of drugs has a remarkable curative effect on erysipelas at all ages and in its varying degrees of severity.

Scarlet Fever. It is of interest that here the results are apparently much less satisfactory, although it should be mentioned that comparatively few reports have been published. Peters and Havard[121] gave their results in 150 cases treated with "Septazine" as compared with 150 controls (56 of which had been given antitoxin). The complications were 35% in the former as against 56% in the latter. They suggested for future trial a combination of antitoxic and drug therapy.

Miscellaneous Oral Infections. Various types of angina including septic sore throat, tonsillitis and various complications such as cervical cellulitis, and other throat infections predominantly caused by hemolytic streptococci, have been treated by these drugs with, as a rule, very encouraging results. Klee and Römer[86] used "Prontosil" in a series of severe septic cases and they have had no fatality in this serious type

of case in the 2-year period during which this drug was available. They gave the histories of 4 typical cases. Schranz,[138] after 2 years' experience with the drug, recommended it in cases of angina both intravenously and as a gargle, using the dissolved tablets. Recknagel[125] gave the history of a case of acute sore throat with arthritic pains which responded promptly. Roth[130] reported the successful treatment of a case of streptococcus septicemia following a sore throat. Tixier and Eck[152] refer to a gangrenous angina cured by "Prontosil." Kramer[89] recommended it for septic throats. Scheurer[134] had an excellent result in a case of purulent angina. Domagk[45c] considered the drug valuable when given by mouth in all types of oral infections because of its being locally absorbed. Jaeger[83] used a concentrated watery solution of "Prontosil Soluble" for local application in angina cases. Long and Bliss[94b] cured 14 cases of tonsillitis and 5 peritonsillar infections with sulphanilamide and its derivatives but lost 2 cases of Ludwig's angina, these patients dying within 20 and 35 hours after beginning the treatment. Two cases of Ludwig's angina which recovered under "Prontosil" treatment are briefly reported by Palmer[119] and by Lyth.[96] Massell[102] studied the effect of sulphanilamide on hemolytic streptococcus infections in the respiratory tract in 6 cases of rheumatic fever and compared these with 6 similar cases given "Aspirin." The results were identical in that recrudescence occurred in 2, adenitis in 1 and death in 1 of each group. A very severe tonsillitis with high fever and inability to swallow which responded rapidly to intramuscular "Prontosil" is reported by Salama.[131] Peters and Havard[121] reported success with "Septazine" in 15 cases of streptococcus tonsillitis, at first thought to be diphtheria, after 48 hours of treatment and in a case of faucial cellulitis from a tooth after 72 hours. Whitby[161] had seven successes and two failures in treating tonsillitis with "Septazine." Kenny[85] mentioned 2 cases as having developed tonsillitis while under treatment for bacteriuria with sulphanilamide. Macnaughton[97] had 2 severe cases of tonsillitis which were cured by intramuscular "Prontosil Soluble." McIntosh[103] reported rather irregular results. Hageman[69a] had excellent immediate results with sulphanilamide in 7 cases of tonsillitis, but hemolytic streptococci persisted in the throat for as long as 5 weeks. Basman and Perley[12] found all of 15 cases of tonsillitis and similar infections did well, but in 9 the clinical improvement was marked under sulphanilamide. Brennemann[25] had favorable results in acute hemolytic streptococcal sore throats in 6 cases: cultures became negative but, a week after the drug was stopped, these again were positive. Smith[145] reported an epidemic of acute tonsillitis in 45 persons from 1 to 45 years old in which 39 were treated. Sulphanilamide was used in 22 (also "Prontosil Soluble" in 4) and "Septazine" in the other 17. Hemolytic streptococcus was cultured from 31, and from 8 less acutely ill *Str. viridans*. The striking effects were in the hemolytic streptococcus cases in that the acute illness was over within 48 hours. There were 5 relapses 2 to 4 weeks later and these again gave a prompt response to the drug. Throat cultures were positive up to 4 weeks after recovery which suggested to him that this treatment may increase the carrier rate. Thirty patients were given the drug prophylactically with apparently good results. Gallagher[57] also had an epidemic of hemolytic streptococcal pharyngitis and 33 patients received treatment with sulphanilamide with the

result that the length of time the throat cultures were positive was significantly reduced, the results seemingly being dependent on the size of the dose and the stage of the infection when it is given.

Otitis media and *mastoid infections* have been treated with sulphanilamide and the results have, as a rule, been excellent with an occasional dramatic cure (Hageman,[69a] Basman and Perley[12]), and sometimes the cases cleared up without a suggested operation being necessary. Hemolytic streptococcal infections did particularly well.

Hemolytic streptococcus meningitis is an infection with such a high death rate that the curative results with these drugs is the most convincing evidence for the high value of this therapy in severe human infection. Gray,[64] in 1935, was able to collect from the literature since 1901 only 66 recoveries. Trachsler *et al.*[154] have added 22 from the literature and one of their own, if we accept 9 in which the hemolytic character of the streptococci is not given (they also tabulate 7 cases of non-hemolytic meningitis), so that the reports from 1901 to 1937 total 89 recoveries out of we know not how many hundreds of fatal cases in which sulphanilamide and its derivatives were not used. The following is a list of cases successfully treated with these drugs, the number of cures when known follows the reference. Caussé[154] (1) the first case reported in February, 1936; Arnold[7] (1) and has since had 4 others[94d] (4), Lucas[95] (1), Vitenson and Konstam[158] (1), Schwentker[141] (3), Anderson[2] (1), Frazer[53] (1), Draeseke and Raynor[46] (1), Weinberg[160] (2), Martin and Delaunay[101] (1), Hageman[69a] (1), Basman and Perley[12] (1), Trachsler[154] (4), Millet[112a] (1), Long and Bliss[94d] (2), they also saw in consultation 9 other patients of whom 8 recovered (8) and Colebrook and Purdie[37] referred to reports by Rouget and Vaidie, Le Mee and Salmon and Neal *et al.* (3). There have been in addition 6 cases of hemolytic streptococcal meningitis in Toronto cured by sulphanilamide. Five of these are reported by Silverthorne and Brown.[144] Thus we have a record of at least 43 recoveries in less than 2 years or practically half the previous record covering over 35 years.

Puerperal infections with hemolytic streptococci are among the most serious of human diseases as well as most variable in their outcome. The difficulty in evaluating statistics has been recognized by all, and in case of successful therapy the favorable results may be due to other factors such as the presence of streptococci of reduced virulence. Virulence as Gibberd,[59] Colebrook[36,38] and others have emphasized is chiefly determined by the evidence of invasion of the organisms into the tissues. The striking results obtained by the use of these new drugs are well represented in puerperal fever and infected abortion cases, especially in those due to beta hemolytic streptococci.

Klee and Römer[86] failed to prevent a fatal outcome in a late case of septic abortion with marked involvement of the perimetrium. Anselm[5] cured 2 cases of sepsis after miscarriage, in one of which there was a pyosalpinx and a Douglas abscess. In 13 other cases of puerperal fever treatment was started at once on admittance with excellent results. He gave "Prontosil" intravenously in the severe cases. Imhäuser[82] was enthusiastic over "Prontosil" therapy for such cases, and gave the histories of 2 puerperal fever cases (one was due to hemolytic streptococcus) and of 4 cases of febrile abortion. Schranz,[138] after a 2-year survey of the use of this treatment, gave as examples of its curative

effect detailed accounts of a case of puerperal sepsis and one of post-abortion sepsis. Fuge[55] in 18 months' experience with "Prontosil" did not lose a case from streptococcus sepsis—his two failures were: in one, death the day after removal of a dead fetus and with streptococcus and staphylococcus metastases already present; in the other, a pure staphylococcus sepsis with widespread metastases. He defined sepsis as the condition when "an infected focus has been formed in the body from which pathogenic bacteria pass out constantly or periodically into the circulation in such a way that subjective or objective disease phenomena are produced by this invasion." He treated 120 women, including 14 cases of sepsis, and obtained excellent results. He emphasized the need of general treatment which by itself has often resulted in cure. Tixier and Eck[152] reported 1 successfully treated puerperal fever case. Vermelin and Hartemann[157] used the drug only in serious cases. They had some difficulty with the available brands of "Prontosil" and give details of 2 cases, 1 having a severe chill after the second injection and in 1 the drug may have caused the death. Sommer[147] also reported a fatal result after the intravenous injection of a solution made up from a new tablet preparation.

The best sustained studies of this therapy in puerperal fever and related infections have been those of Colebrook and his colleagues, and a review cannot do justice to the careful and unprejudiced analyses they have made of their results. Their first trial[36a] of "Prontosil" was made after a very full study of the effects of the drug in experiments on animals, and because of the high virulence of the streptococci demanded under these conditions they were not very hopeful that it would be successful in the human. They treated 38 cases and reduced the death rate to 8% from one of 26.3 in the preceding 38 cases and from an average of 22% over a 4-year period. They noted that the streptococci could be cultured from the discharges of treated cases just as often and for just as long as had been found in their previous cases. In a second report[36b] 26 further cases with no deaths are considered, but the statistical study of the different types of infection in the then total of 64 cases cannot be profitably reviewed. They believed that in at least 20 or 30 of these the course of the infection had been profoundly modified by the drug. The most striking results seemed to be in the fall in the death rate (4.7), especially from peritonitis, failure of the spread of the infection to the cellular surrounding tissues, the rapid drop in the temperature and the cure of 9 out of 12 cases with positive blood cultures. Their last report[37] covered their results with sulphanilamide in 100 cases with 8 deaths (a note refers to 15 additional successful cases giving a death rate for hemolytic streptococcal infections of 7%). In the final total of 199 cases of hemolytic streptococcal infections treated by either drug the death rate was 5.5 as compared with 22.8 in the 5 previous years. They believed "Prontosil" is slightly better than sulphanilamide. Another excellent report covering some of these same cases is that by Gibberd,[59] who showed a death rate of 4.5 for the 157 cases treated between January, 1936, and March, 1937. This report should be read in its entirety for the detailed analysis of this valuable material.

In the interval between these reports, Ley,[93] who had used "Prontosil" given by various routes for some 2 years, gave his results for

37 cases treated therapeutically with four failures (given in detail) and for 42 cases in which it was used prophylactically before operation or other interferences usually associated with a later pyrexia. Of these latter cases, 35 did not develop fever and only 1 died. He was very enthusiastic but emphasized that treatment should be started before 48 hours after a rise in temperature to obtain the best results. Long and Bliss[94a] had success in a case of infection after abortion with a pelvic peritonitis. Foulis and Barr[49] used sulphanilamide in 22 severe septic cases (11 septicemia, 4 peritonitis and 7 others—all hemolytic streptococci) with a rapid and striking fall in temperature and general improvement. In 4, "Prontosil Soluble" was also given. One patient died, making a death rate, from a total of 70 septic cases in hospital in a 3-month period, of 1.4%. Peters and Havard[121] treated a case of puerperal fever and infiltration of the broad ligament by "Septazine" with a prompt curative result.

Hemolytic Streptococcus Bacteremia Other Than Puerperal. There are a number of reported cases which merit reference. Roth's case,[130] already mentioned, followed an oral infection and was cured under "Prontosil." Lampert[91] had 2 successful cases after erysipelas. Long and Bliss[94a] treated 3, 1 of these died 7 hours after treatment was commenced, had been ill for several days and was practically moribund. The other 2 recovered. Robinson[126] was successful in treating a very severe case with "Prontosil" and although antiserum was also given, the cure is most probably due to the drug. Whitby[161] treated 5 cases with "Septazine" and had one failure. McIntosh[103] had 2 successful cases treated with sulphanilamide. Mitchell and Trachsler[113] treated 3 children, 1 died and 2 recovered, but for only 1 of these was the drug given credit. Carey[34] was successful in 5 cases and Basman and Perley[12] in 1. Brennemann[25] reported a case in a child with an infected hip and hemolytic streptococci grown both from this site and from the blood. The child was recovering at the time of the report. Macnaughton[97] treated with success by "Prontosil" a very severe bacteremia from an infected ear with repeated positive blood cultures.

Hemolytic streptococcus peritonitis cases other than puerperal have been occasionally treated. Long and Bliss[94a] failed in a 4-month-old infant, as did Basman and Perley[12] in a baby; and Bernstein[17] was unsuccessful in a boy of 6 who also had a bacteremia. Brennemann[25] cured a boy of 9 and McIntosh[103] reported 3 cases of "primary" peritonitis in infants and had in 2 a favorable and in 1 a questionable result under sulphanilamide treatment.

A variety of other hemolytic streptococcal infections have responded to this chemotherapy. Gmelin[61] gave a short note on his excellent results in 2 cases of empyema after giving "Prontosil" by mouth. Schranz[138] injected "Prontosil" about the site of operation as a prophylactic. Domagk[45c] treated a severe infection in his own daughter. After a needle which had entered her hand was removed a phlegmonous inflammation spread to her arm. When repeated incisions failed to stop the spreading infection she was given a series of treatments with "Prontosil" by mouth and rectum, and a complete cure was obtained. Jaeger[83] used "Prontosil" chiefly as a saturated solution in equal parts of alcohol and acetone as a local treatment in a great variety of conditions, such as cuts, street wounds, and to replace iodine in surgery. Long

and Bliss[94b] had success in 2 cases of streptococcus osteomyelitis. Peters and Havard[121] were able to stop a hemolytic streptococcal pleurisy following an influenzal pneumonia after only two aspirations by means of "Septazine." Brown[26] reported the marked benefit he obtained by the use of "Prontosil" given intrapleurally in 2 patients with streptococcal empyema after influenza and who were in extremely poor condition. Bensley and Ross,[14] in a special study on methemoglobinemia in sulphanilamide therapy referred to a case of hemolytic streptococcal pneumonia, cyanotic on admission, and who developed methemoglobinemia so that an estimated 22% of the total hemoglobin was involved. In this case the anoxemia was aggravated by this condition. McIntosh[103] had a favorable result in an infant with a streptococcal pneumonia. Hageman[69a] listed 5 cases in their pediatric service in 3 of whom empyema was present before treatment, 2 of these required thoracotomy and all recovered. Basman and Perley[12] had an empyema in a baby cured by thoracotomy and the drug, and a 1-year-old boy, with an empyema 10 days after a pneumonia before admission, died in spite of the drug. Smith[145] reported 2 cases of acute septic arthritis of the hip, both gravely ill, which recovered under treatment with these drugs. Long and Bliss[94b] listed 3 cases of traumatic orbital cellulitis with a hemolytic streptococcus infection, all recovered. They had also 3 recoveries in streptococcus urinary cystitis. Colebrook and Purdie[37] mention an unreported case of extensive suppuration in the cellular and bony tissues of the pelvis, not of puerperal origin, in which a dramatic result followed sulphanilamide therapy. A particularly interesting case is the one reported by Purdie and Fry[124] of a chronic streptococcal infection with many abscesses and a history going back almost 3 years to a puerperal infection. The streptococcus found at the time of sulphanilamide treatment was the same serologic type as that grown from the primary infection. After 19 days' treatment in hospital with the drug by mouth and local irrigation of the abscesses and use of sulphanilamide powder, the discharge lessened, the wounds were healing, the cultures became sterile 4 days after and a complete cure was effected. This, then, is a short account of the situation in respect to the treatment of hemolytic streptococcal infections in man and it must be looked upon as indicating a new and highly encouraging advance.

Staphylococcal infections are different in the type of lesion produced, the relatively low invasive character of the organism and in many other ways. The experimental work with these chemicals preceding their application to human infections was, as outlined above, rather meagre and unsatisfactory. Nevertheless the drugs have been given many trials and at times with surprisingly good results. The first reference to "Streptozon" ("Prontosil") in the literature[94d] reported a marked chemotherapeutic effect in a case of generalized staphylococcal infection. Schranz[138] treated with success an upper lip furuncle which contained both streptococci and staphylococci with "Prontosil" and also a case of the same kind with only staphylococcus as well as another pure staphylococcal skull wound. Anselm,[5] having had a prompt response and a rapid cure in a mixed infection of streptococcus and staphylococcus in a patient with high fever after an abortion at the third month, treated 2 puerperal pyretic patients with a pure staphylococcal parametric infection with equally good results. Schreus[139]

found "Prontosil" although not as selective as in streptococcal infections had nevertheless a definite effect. He treated a young child covered with staphylococcal abscesses over most of the body, a positive blood culture, and so seriously ill that death was expected daily. "Prontosil" was given by mouth twice a day, the abscesses softened, the temperature fell and the patient recovered. However, in cases of furunculosis his results were not satisfactory. Imhäuser[82] had failures in 2 septic cases. In one there were intracranial foci and death from meningitis. In the other, despite care and prolonged treatment with "Prontosil," death followed with leukopenia, in which no leukocyte reaction could be induced even by a turpentine injection. (Was this possibly a case of leukopenia from the drug?) Tonndorf[153] used "Prontosil" in phlegmons, furunculosis and similar infections and believed it did good, but found it impossible to draw definite conclusions. Meyer zu Hörst[111] considered the drug shortened the duration of furunculosis in infants. Jaeger[83] used the preparation referred to above locally in cases with furuncles, carbuncles and a variety of similar conditions and claimed to have had excellent results. Long and Bliss[94a] treated with sulphanilamide a resistant case of impetigo from which hemolytic streptococcus and staphylococcus were cultured with the disappearance of the streptococcus within 4 days and a slow clearing of the lesions. Walther[159] mentioned 2 patients with severe urosepsis, one with *S. aureus* alone and a positive blood culture, the other with this organism and *E. coli* that responded to a combined therapy of "Prontosil" intramuscularly and sulphanilamide by mouth. Basman and Perley[12] lost 2 cases with staphylococcemia, and in an osteomyelitis case with a double infection which recovered noted that the streptococcus disappeared first, and believed the drug may have helped. Colebrook and Purdie[37] had 3 puerperal staphylococcal infections of which 2 were cured.

Meningococcus meningitis is obviously an infection which might be expected to respond to this new therapy following the experimental work and the remarkable results in the streptococcus cases. Proom[123] believed the bacteria are carried to the meninges by the blood. Schwentker, Gelman and Long[142] treated with sulphanilamide 10 cases of meningococcus meningitis and 1 of meningococcemia. The drug was given subcutaneously and intraspinally and the cerebrospinal fluid became sterile in 3 days or less. In the 1 fatal case, the desperately ill patient had had a sterile fluid for 3 days and the cell count had been reduced to 158 when he died from a pneumonia on the fifth day. McIntosh[103] reported 2 cases of meningitis with bacteremia, which treated with specific antiserum and sulphanilamide, were both cured. Mitchell and Trachsler[113] had 3 recoveries following a combined therapy of antiserum, antitoxin and the drug intraspinally and by mouth. In one of these the spinal fluid had given cultures for 10 days but became negative the day after the first intraspinal injection of the drug. Bernstein[17] reported 2 recoveries, in 1 receiving only a transfusion of blood the recovery was slow, in the other both antiserum and drug were given and recovery was somewhat more rapid. Carey[34] treated 5 cases with the drug alone given by various routes. The blood cultures were positive in the 3 in which the blood was cultured. All recovered without sequelæ. Basman and Perley[12] report 2 recoveries, in 1 a single injection of 15 cc. antiserum was given in addition to the drug therapy. Brennemann[25] treated 1 case with 3 large doses of antitoxin, "Prontosil Soluble"

and sulphanilamide, the case recovered but the value of the drugs was of course uncertain. Schwentker, in the discussion of a paper by Bliss and Long,[19b] referred to having used sulphanilamide in 52 cases of meningococcus meningitis with a mortality of 15% as compared to a 30% mortality among patients treated with antiserum in the same hospital during the same epidemic. The evidence from the above results and the experimental work would suggest that a combined sulphanilamide-antiserum therapy is perhaps the best treatment.

Gonococcus infections have been much more widely treated with sulphanilamide and its derivatives than the reports in the literature would indicate. This is suggested by the relatively high percentage of cases of gonorrhea mentioned in reports in which secondary effects, particularly in the skin, are being considered.

Domagk[45a,b] compared the chemical structure of "Prontosil" and a number of chrysoidine compounds, which under trade names have long been recommended for urinary tract infections and for gonorrhea. The new drug was therefore given a trial by many. Schreus[139] did so, but found it unsatisfactory, although he obtained a definite action on the fever and local lesions, especially in acute cases. I have not seen his promised report covering his later experience. Becker[13] recorded a complete failure for "Prontosil" therapy in gonorrhea. Dees and Colston[42] in a preliminary report gave their results with an exclusive sulphanilamide therapy in 19 cases. The active discharge rapidly disappeared after the following days (cases in parentheses): 1 (3), 2 (7), 3 (2), 7 (2)—in the others more irregularly. The smears became negative in most in the first week after treatment. In 5 or 6 the response was slower and in 3 or 4 there was little if any benefit. They also had good results in chronic prostatitis. They found that the drug checked the spread and prevented complications, but they fully realized the need for a longer and more extensive experience. In 28 additional cases (making a total of 47) they had equally good results; there was never a progression of the infection even in the cases showing no response to treatment. Grütz[68] carried out clinical tests, with a number of preparations not on the market but supplied from Domagk and his associated chemists, in which it was demanded that the drug given *per os* be readily absorbed and reach the various sites where the gonococcus grows as an epithelial parasite, particularly in the lumina of the urogenital glands, that local and other treatment be stopped during the tests and that the results must be evident in a few days. Under these conditions "Prontosil" showed little activity, sulphanilamide had definite effects in reducing the numbers and causing the disappearance of the gonococci, but this was not constant and the disease sometimes progressed so that new preparations were tried. Three of these D.B.90 (Uleron or Diseptal A), D.B.87 (Diseptal B), and D.B.32 (disulphanilamide or Diseptal C) were found to be superior to sulphanilamide. He reported his results in 36 cases of both sexes with acute and chronic disease showing direct curative effects in 24 and failures in 12 (of which 9 were complete). Some of his failures he thought might have been due to poor absorption resulting from alkaline changes in the gastrointestinal tract. There were a number of secondary reactions from the drugs but none of these were serious. He considered the drug Diseptal B (D.B.87) the best, so that it is not clear why Diseptal A (D.B. 90) was chosen for the later more extensive study. Hageman,[69a] using

sulphanilamide, reported obtaining negative smears after 36 hours in a case of ophthalmia, and one good response out of three in vaginitis cases. Ballenger[8] had better results in a number of resistant cases by a combination of thermotherapy and sulphanilamide and strongly advocated its use in these resistant types of infection. Walther[159] referred to having treated 8 cases with sulphanilamide and regarded it as a useful drug. Felke[47] used D.B.90 (Diseptal A) and in a note gave it the name "Uliron." The most interesting point in his report is the stress he laid on the necessity of the tissue reactions in determining the final and complete cure of gonorrhea whatever the treatment may be. He considered that at the fifth to sixth week in untreated gonorrhea a stage is reached when a few bladder washings (Janet's original technique) will bring about a cure. He had some 50 case histories supporting this view, and even an epididymitis was believed to bring about more rapidly this "preparation for cure." The situation is different in the female in that involvement of the adnexa interfered with cure. He had given up the local use of silver preparations in all acute cases, at least in male patients. Reference is made to the use of "Prontosil" by many clinicians, but, with the exception of Linser, the only result was a rapid clearing of the second urine specimen but not a permanent cure. He found in 50 cases treated with "D.B.90" ("Uleron") that he could not abort acute cases in that, although the signs and symptoms disappeared in 1 to 3 days, the gonococci remained, because time is needed for the biologic defense. In ambulatory cases he allowed 2 weeks to elapse before giving the drug, and had even better responses in older infections and in those with epididymitis. His clinic treats mostly male cases, but he said that females were rapidly cured if the tubes are not involved. He found the blood of treated cases did not inhibit the gonococcus.

Uleron (the Winthrop Chemical Company's name for this drug) (also known as Diseptal A) is not being marketed in North America, but has been made available in a number of medical centers for clinical trial. The therapeutic results have been remarkably good, but because of certain undesirable effects (which have not been reported in the literature and which were unpredictable from the favorable biologic findings) the drug has been withdrawn for further laboratory investigation. Orr[118] treated 104 male and 30 female cases of gonorrhea, mostly ambulatory, with sulphanilamide given by mouth and used no local treatment with a resulting cure in 87.3%. Among the 17 cases in which the treatment failed, none showed any beneficial effect at any time. Keefer, in discussing a paper by Bliss and Long,[19b] said that when sulphanilamide is added to whole blood or plasma these become bactericidal for the gonococcus, as was also the case with the blood of treated cases. He reported success in sterilizing the synovial fluid after giving the drug by mouth in several patients with gonococcal arthritis. Cokkins[35] published his early results after treating 250 cases with sulphanilamide *per os*. He considered it an effective remedy for gonorrhea and most of its complications, and that all the clinical cures could be made permanent if the drug is continued for 2 to 3 weeks. It must also be given in potentially toxic doses. Chronic urethritis and prostatitis cleared up at least as rapidly as did the acute infections, but a small group completely failed to respond.

Pneumococcus infections have been treated in only a few cases. Heintzelman[75] had 19 cases of Type III pneumonia, 9 of whom received sulphanilamide and 10 did not. Seven of the treated recovered and only 2 of the untreated. From 33 untreated cases in Pittsburgh only 9 recovered, giving a 75% death rate, as compared with 22% under the drug therapy. Basman and Perley[12] reported a Type V pneumococcus meningitis in a child which recovered under this therapy—the first proved case of pneumococcus meningitis in their hospital which was not fatal. They also had a second case of Type III infection in which the drug failed and another which died 15 hours after admittance. A Type V as well as an untyped mastoid case and a Type III brain abscess case all died. Of 2 cases of Type I empyema 1 recovered and 1 died, while a Type II pneumococcemia recovered. Millet[112b] had a successful result in a Type III pneumonia patient. Keith, in Toronto, had a case (not yet reported) of Type III pneumococcus meningitis which recovered promptly under sulphanilamide therapy. There are other scattered references to the use of these drugs in middle ear and other infections but the results were rather irregular.

Kidney Infections. These drugs have been and are being rather widely used in kidney infections with *E. coli*, and other bacteria. Klee and Römer[86] did not find "Prontosil" of any value in clearing up the bacteriuria in an epidemic of typhoid fever. Imhäuser[82] gave details of 4 cases of *B. coli* pyelocystitis which he cured by "Prontosil" therapy. Holzmann[78] warned that pyuria in children tends to show defervescence and improvement at 7-day periods, a fact which he held as important in prognosis and in preventing therapeutic hyper-enthusiasm. Huber[80] quoted Temming as the first to use "Prontosil" in 3 cases of pyuria in children, and Pernice, who had good results in 18 coli-pyuria cases in the young. He tabulated his own therapeutic results in 14 young females showing the clearing of the urine, the drop in the fever and the favorable outcome in both acute and more chronic cases. Hofmann[77] quoted Unshelm as having had a large measure of success in similar types of cases. Long and Bliss[94c] referred to the slow excretion of sulphanilamide through the damaged kidney as a danger in such therapy. Borst[22] treated 13 cases of coli-pyelocystitis with "Prontosil," and in 5, toxic symptoms were produced, and in 1 of these a fatal agranulocytosis suddenly developed. Kenny[85] reported 46 cases of *E. coli* infections of the urinary tract in all of which there were clinical symptoms which disappeared under sulphanilamide treatment, and the urine became sterile usually in a few days. The urine of the treated cases shown to contain sulphanilamide both free and combined was found to be bactericidal to a large number of bacteria isolated from the cases. The different strains varied in their sensitivity to the drug but the patients having resistant strains responded to treatment just as well as those with sensitive strains. Mitchell and Trachsler[113] had a case of coli-pyelitis which was improved by the treatment, Hageman[69a] a pyuria which cleared completely in 48 hours, and Basman and Perley[12] had a similar result but in another the child was sensitive to the drug. Helmholz[76] showed that sulphanilamide causes the excretion of an alkaline urine which is strongly bactericidal for most of the organisms found in urinary tract infections—the chief exception being *Str. fecalis*—and that this drug therapy is successful even in cases of urinary insufficiency.

Mellon and Shinn[107] were able to demonstrate bacteriostatic effects in urine if urine is used in the media and the diluent in place of broth. Sulphanilamide 1:10,000, a concentration often found *in vivo*, was particularly effective. Walther[159] considered that sulphanilamide will occupy an important place among the urinary antiseptics. Barer,[9] in a case of typhoid bacilluria unaffected by hexamine and ammonium mandelate, found the bacilli disappeared permanently from the urine under sulphanilamide treatment.

Miscellaneous Diseases.—There are many reports published on the use of these drugs in all manner of diseases. In acute rheumatism, Veil and Recknagel[125] reported a drop in temperature and in the sedimentation rate, McQuarrie[105] had a case which greatly improved under sulphanilamide, and there are other references in which the results are very uncertain or fully negative. Bingold[18] reported an apparent cure in a case having the Ebstein type of remittent fever, lymphopenia and eosinophilia and enlarged lymph nodes which, however, was not proved but was suspected as being Hodgkin's disease. Skin diseases of various kinds have seemingly responded. Jaeger[83] had a good result in a case of lupus erythematosis, the progress in a case of psoriasis was stopped, and "Prontosil" in alcohol and acetone was particularly effective in weeping eczema and in dermatomycosis. Bohlman[21] successfully treated 3 cases of gas gangrene with sulphanilamide and a combined gas bacillus antitoxin. In one of these cases the result was truly dramatic. He suggested that part of the effect may have been due to the prevention of the symbiotic growth of the streptococci.

Little need be said in reference to specificity after reading this review. The problem arises as to whether the kind of tissue reaction, dependent in many cases on the virulence and type of infecting organism, favors the action of these drugs or whether it is a peculiar bacteriostatic effect with variable results affecting in different ways the invasive and pathogenic characters of the bacteria. Hofmann[77] showed that "Prontosil" reaches a sterile turpentine abscess during the active stage, but after the abscess has ceased to progress the dye is no longer found in the pus. This may be also true for the other drugs.

Pharmacology and Secondary Reactions. However effective in therapy this group of drugs may be, and their value is indeed no longer questioned, yet they are all more or less toxic to animals and man. Secondary reactions, some trivial, some serious, are extremely common when they are given to patients ill with various diseases. Weese and Hecht are quoted by Domagk,[45] Hörlein[79a] and others as having shown that "Prontosil" is a very indifferent drug in animals. It had in rather large doses little demonstrable effect on the blood or the functions of the various organs, but with clinical experience and more extensive studies in animals and man the belief that this and similar drugs are innocuous has been rudely shaken.

Evidence for the toxic effect accumulated. Domagk[45a] observed that 0.5 gm. per kilo of "Prontosil" was well tolerated by mice and dogs *per os*, but that the cat only tolerated 0.2 gm. per kilo. Tréfouël[155] found sulphanilamide less toxic than "Prontosil" in animals. Bloch-Michel[20] confirmed this and also noted the lower toxicity of "Septazine." Buttle[30] confirmed Tréfouël's findings. In a comparative study of

diaminodiphenyl sulphone, Buttle[32] found this drug 10 times more toxic than sulphanilamide in single doses and 20 times in daily doses in infected mice, but not for normal rabbits or monkeys. It was, however, curative in mice in a dose 100 times less. Proom,[123] in his experiments, used sulphanilamide suspensions subcutaneously and orally, but not intraperitoneally because of its toxicity by the latter route. Whitby[161] reported on the relative toxicity of "Prontosil Soluble," sulphanilamide, "Septazine," "Soluseptazine" and other compounds, finding "Septazine" relatively non-toxic. Halpern and Mayer[71] found "Prontosil" and sulphanilamide slightly but not negligibly toxic, and that they affected the nervous system in the order given. "Septazine" was definitely less toxic. The therapeutic coefficients they gave as $\frac{1}{1}\frac{}{0}$ for sulphanilamide, $\frac{1}{4}$ for "Prontosil" and $\frac{1}{4}\frac{}{0}$ for "Septazine" in the mouse and other animals. Hawking,[74] in a pharmacologic study of sulphanilamide base, reported that in acute poisoning the central nervous system was involved but the other organs showed no evident change. In a 1:1000 solution it had no effect on the rabbit intestine or guinea pig uterus *in vitro*, and a 1.5% solution did not change the blood pressure in the cat and dog. Large doses in the peritoneal cavity had practically no irritating effect. The toxicity tests by Rosenthal[128] on sulphanilamide and other compounds have already been mentioned. Hageman[69b] studied the pathological lesions in mice produced by 50 mg. of "Prontosil Soluble" or 20 mg. of sulphanilamide given parenterally each day for 2 weeks. There were no reactions noted to the first drug, but with the latter severe nervous symptoms occurred. The finding of hemosiderin in the spleen of the sulphanilamide-treated animals suggested increased blood destruction. Barlow[10] found the M.L.D. for "Prontosil Soluble" and disulphanilamide *per os* was over 40 gm. per kilo in mice, that for sulphanilamide given in 50% acacia was 6.25 gm. By the subcutaneous route the M.L.D. for "Prontosil Soluble" was 6 to 8 gm. and for sulphanilamide 2.75 to 4 gm. per kilo.

Toxicity in Man. In this brief consideration of toxicity in animals it may be noted that many factors influence the results, and that the animal species differ in their reactions. It is, therefore, only in man and almost exclusively when he is suffering from some infection that useful facts about the actual toxicity of these drugs in human therapy can be obtained. The literature on this phase of the subject covers the vast majority of all the reports. I will attempt very briefly to group these under a number of headings.

Blood. Many have realized the potential danger suggested by the presence of the benzene nucleus in most of these drugs. Cyanosis is one of the most common of the secondary effects. Its explanation is far from clear. Colebrook and Kenny[36a] first called attention to the occurrence of sulphemoglobinemia in "Prontosil" treated cases. In 3 cases of cyanosis in which the respiratory rate was unaffected this condition was associated with the use of magnesium sulphate. Southworth[148] noted that cases treated with sulphanilamide showed a drop in the CO_2 combining power of their blood plasma, and in 2, clinical acidosis developed after large doses of the drug. Long and Bliss[94b] reported 4 cases with slight cyanosis and rapid breathing which were diagnosed as acidosis, and also 3 cases of sulphemoglobinemia. None of their patients had had saline cathartics or laxatives. Foulis and

Barr[49] had 2 cases with slight cyanosis, in only 1 of which had magnesium sulphate been given. Thomas'[151] case of streptococcus meningitis developed under "Prontosil" an extreme cyanosis with dyspnea. Schwentker[141] had a fatal case in which clinical acidosis, dyspnea, reduced CO_2 combining power of the blood and slight cyanosis developed. Long and Bliss[94c] found cyanosis in three-quarters of their patients treated with sulphanilamide, but they did not consider that it warranted stopping the treatment. In practically all their patients a fall in CO_2 combining power of the blood occurred and they recommended giving sodium bicarbonate to prevent this, and sodium lactate was used to cure and prevent acidosis. Frost[54] reported a case dying of respiratory failure in which cyanosis developed after 2 5-cc. injections of "Prontosil" and 12 tablets of "Septazine" in 2 days. Sulphemoglobin bands were found in the blood at autopsy. Magnesium sulphate had not been used. Discombe,[44] having discovered a case of sulphemoglobinemia with extreme cyanosis after sulphanilamide therapy, studied a series of 7 cases and found the former in 6 receiving over 5 gm. of the drug, and in 3 of these also a marked cyanosis. The association with magnesium sulphate is indicated in all 6 cases, in 1 the salt having been used only as a skin dressing. In 1 case, despite long use of the drug in the absence of sulphate, there were no abnormal pigments in the blood. The danger in anemic patients is suggested and the need of spectroscopic blood tests is stressed. Paton and Eaton[120] studied this problem in some detail on 20 cases and concluded that magnesium sulphate given with sulphanilamide usually gives rise, often rapidly, to sulphemoglobinemia, and without this salt the drug is well tolerated, but in some cases methemoglobinemia develops. The latter condition clears rapidly after drug is withdrawn and giving oxygen is helpful, the former may last for as long as 6 weeks and oxygen is of little value. Peters and Havard[121] had no case with cyanosis or other clinical signs suggesting sulphemoglobinemia from 215 cases treated with "Septazine." Borst[22] had a patient who became seriously ill under "Prontosil" therapy, with dyspnea and Cheyne-Stokes respirations, but without cyanosis. Whitby[161] had no case of sulphemoglobinemia in 20 cases under "Septazine." Kenny[85] had 7 cases from 47 treated with sulphanilamide in which there was methemoglobinemia without symptoms. Snodgrass and Anderson[146] reported only 2 cases of cyanosis from 160 treated with "Prontosil" and in these treatment was continued. Bensley and Ross[14] showed that methemoglobinemia is not necessarily related to dosage of sulphanilamide. They studied the hemoglobin (total, oxygen carrying and met- compounds) in a series of cases and indicated that in certain cases anoxemia may be aggravated by an anemia and methemoglobinemia. The need of spectroscopic tests was indicated in a case of pneumonia. Hageman[69a] reported cyanosis associated with methemoglobinemia in about half of their treated cases. Marshall and Walzl[99] having noted cyanosis in a large number of cases tested the blood of 7, and found in clinical cyanosis there may not be a decrease in oxygen-carrying capacity of the blood nor the presence of non-functional iron pigment (methemoglobin). Further they showed that "dark bloods" have no more than traces of this pigment, so that it can be eliminated as the cause of the cyanosis in at least 5 and probably was not the only cause in 2 other cases. They suggested as an explanation a black oxi-

dation product of the drug staining the red blood cells. Archer and Discombe[6] concluded from their study that sulphanilamide, like phenacetin and methylacetanilide and other similar drugs, can form sulphemoglobin or methemoglobin in the blood. They are derivatives of aniline and nitrobenzene compounds and act as catalysts. The drugs with $C_6H_5N<$ in their structure are apt to cause these changes. How methemoglobinemia is formed is unknown they said, but sulphemoglobinemia they believed is formed from excess hydrogen sulphide produced by bacteria in the bowel, and that in the presence of these drug-catalysts the sulphur pigment is formed with relatively smaller amounts of the sulphide. The effect of magnesium sulphate they considered as due to the more fluid bowel contents favoring bacterial growth, and that low residue diets can to a degree control this sulphide production. They reported 3 cases, cyanotic after "Septazine" therapy and not on a low residue diet, in 2 of which sulphemoglobinemia was present. Colebrook and Purdie[37] examined the blood in 53 of 58 cyanotic patients receiving sulphanilamide and found sulphemoglobinemia in 13, methemoglobinemia in 24, and both in 8.

Anemia is a possible secondary effect and in infected cases is confusing as to its etiology. Long and Bliss[94c] had 2 severe acute anemias in patients receiving sulphanilamide. Harvey and Janeway[73] reported acute hemolytic anemia in 3 cases receiving large doses of sulphanilamide, which cleared up after the drug was stopped. Kohn[87] also had a case of this kind and considered that it must have been due to a predisposition. It is of interest that Jaeger[83] found "Prontosil Soluble" prevented to a degree coagulation of the blood, and that in Frost's[54] fatal case after a total of 10 cc. "Prontosil" and 12 tablets of "Septazine" the blood at autopsy is reported as having been dark and fluid.

Although several writers refer to the reduction in the number of leukocytes as a favorable sign in this kind of therapy, a few cases have occurred in which leukopenia was present. Massell[102] had such a case in which the white cell count fell to 3700 under sulphanilamide but slowly rose after withdrawing the drug. Plumer,[122] in a patient with subacute bacterial endocarditis (*Str. viridans* in the blood), found a sudden fall in the cell count from a previous 21,000 to 400 and the complete absence of neutrophils, the patient died with the signs and symptoms of agranulocytosis. Borst[22] had a case with a history of bleeding gums, and other hemorrhagic disturbances some years before, who while being treated with "Prontosil" died following a rather sudden drop in the white cell count. Young,[162] realizing that because of the types of infection treated by these drugs the presence of agranulocytosis if caused by the therapy could be readily overlooked, undertook the study of the leukocytes in treated cases. He reported a fatal case of agranulocytosis which suddenly developed under sulphanilamide. McIntosh[103] had a case of granulocytopenia in a baby under small doses of sulphanilamide. These are rather rare occurrences but serve as a warning against the possible harm of these compounds in certain individuals.

Effects Seen in the Skin.—The skin rashes and other cutaneous reactions are rather common secondary effects during the use of many of these drugs. An analysis of these should be made by an expert dermatologist so that we may learn more accurately the site of these dis-

turbances and whether they are due to changes in the blood, the walls of the capillaries or in other tissues. The staining of the skin with the dye compounds is commented on in most of the early reports, but very little is said about unpleasant reactions.

Sensitivity to Light.—Gmelin[61] as well as Schreus[139] specifically mention that "Prontosil" did not increase the light sensitivity of the skin. However, under sulphanilamide therapy Menville and Archinard[109] had 4 cases in which after exposure to the sun such a sensitization was shown by severe reactions. Frank[51] reported 2 similar cases, Newman and Sharlit[115] 4 and in a note referred to 6 others, and Grosjean[65] 1. It is of interest that of the 11 cases in the last group with histories available, 7 were cases of gonococcus infections, and in a large proportion of the cases with cutaneous reactions to be referred to below this type of infection seemed to predominate. Of course, such ambulatory patients are subject to sun exposure, which may explain the incidence. Colebrook and Purdie[37] and many others have never seen this phenomenon.

Skin Rashes. Peters and Havard[121] had only 1 case with a macular rash out of 215 "Septazine" treated patients. Ley[93] reported only a slight and temporary urticaria in 2 from numerous cases treated with "Prontosil." Grütz,[68] using drugs of the "Uleron" series, recorded skin disturbances in 7, and Felke[47] with "Uleron" had only 1. Schwentker and Gelman[140] studied particularly the sulphanilamide rash. They had 10 cases from a total of 180 treated persons with an early rash, but in about half of all the cases it developed later (10 to 14 days). It was morbilliform in appearance and faded whether or not the drug was withdrawn, but more slowly if the drug was continued. It was often associated with a fever. Hageman[69a] reported that in 1 of every 6 sulphanilamide cases fever occurred, and in half of these there was a morbilliform rash. Goodman and Levy[63] had 2 cases of toxicodermatosis in gonorrheal patients, in 1 there were hemorrhagic lesions and they believed it might have an allergic basis. Schonberg[137] recorded a case in which a purpuric and scarlatiniform eruption, apparently as an allergic reaction, followed the use of sulphanilamide. Salvin[132] described, in a patient with acute urethritis, a hypersensitivity to this drug with urticaria and other lesions of a type so severe that this therapy had to be given up. Colebrook and Purdie[37] never observed morbilliform rashes nor were the mild skin reactions ever associated with fever. Finney[48] had an example of dermatitis medicamentosa in a recurrent gonorrheal case which was so severe that hospitalization was necessary. Myers[114] reported a severe exfoliative dermatitis with edema, purpura, fever, eosinophilia and other changes in a patient who had taken 88.3 gm. of the drug for a gonorrheal prostatitis. No attempt has been made to comb the literature, but enough has been mentioned to indicate the potential danger. It may appear that sulphanilamide is particularly responsible for most of these reactions, but this is probably due to the vastly greater numbers being treated with this drug.

Fever occurs in some cases and this adds a difficulty to the differential diagnoses. Long and Bliss[94c] found that "Prontosil Solution" produced but one toxic effect, namely, fever. Hageman and Blake[70] reported its occurrence in 21 of 134 cases under sulphanilamide treatment and compared the associated reactions to those of serum sickness.

Fever is mentioned by Recknagel,[125] Roth,[130] Grütz,[68] Felke,[47] Colebrook[36,37] and a number of other authors.

The gastro-intestinal tract shows certain reactions to these drugs. The preparations used for oral administration, the relative irritability, the psychic effect where the dye drugs are used, the acidity in the stomach all need to be considered. Although the pharmacologic studies showed no evidence for any effect on smooth muscle, yet the occasional diarrhea (Recknagel,[125] Bucy[28]), the desire to defecate (Colebrook and Kenny,[36a] Long and Bliss[94a]), and the frequently reported nausea, all indicate that in sick patients the effects may be somewhat different. The report of Schneider[136] on the unexpected violent reaction in the smooth muscle organs of the uterus, rectum and bladder in his case is of some interest.

The kidney seems relatively resistant, although Colebrook and Kenny[36a] reported some effects, Whitby[161] found a certain irritation from "Prontosil Soluble" and sulphanilamide, and Mitchell and Trachsler[113] had a case of hematuria after large doses of these two drugs. The most unfortunate series of deaths following the use of Elixir of Sulphanilamide-Massengill need only be referred to.[1] The kidney damage was shown to be due to the diethylene glycol content of the mixture, and the sulphanilamide was in no way responsible for the tragedy.

The nervous system, although involved in the acute toxic effects of these drugs in animals is practically never affected in man. The occasional case of dizziness, headache and similar symptoms are probably of circulatory origin. Whitby[161] merely mentioned a case that he had heard about with effects in the nervous system. Bucy's[28] case of optic neuritis is a most exceptional incident, and here the ferrous sulphate given with the sulphanilamide may have been in part responsible.

In summing up these secondary reactions it may simply be repeated that the chemical makeup of these drugs suggests possible harmful effects. The growing experience in treating patients has shown the need of appreciating the dangers potential and demonstrable, but these should not cause the splendid results of this type of therapy to be forgotten.

The Mode of Action of These Therapeutic Drugs. We have only the most superficial knowledge of the actual therapeutic mechanism of such well established and pragmatically effective drugs as salvarsan and its related compounds. Nevertheless it is evident to all that the knowledge of the mode of action of the drugs we are considering would be of immense help.

Direct Action on the Bacteria. It was evident from the very beginning that we were dealing with a type of action in these drugs rather different from that recorded for most previously used antibacterial chemicals. The original experiments of Domagk[45a] were based on the idea that chemical compounds with no appreciable bactericidal effect *in vitro* might be active against bacteria *in vivo* and that is how it turned out. Nevertheless, a great many attempts have been undertaken to demonstrate some direct destructive action and it would appear that these have failed to clearly show such an action. It is recognized that comparatively slight changes in culture media will prevent growth of many

bacteria and that bacteriostatic effects are soon followed, as a rule, by the death of the organisms. It would therefore be of little value to divide sharply bactericidal from bacteriostatic effects.

Levaditi and Vaisman[92a,b,c] confirmed the work of Domagk in that they were unable to demonstrate any bactericidal action of "Prontosil" *in vitro* by various technical procedures, nor did they find any loss in mouse pathogenicity of streptococci even after prolonged contact with "Prontosil" in the test-tube at 37° C. Their assistant Deutsch showed that streptococci would not grow in either chicken plasma or embryonic juice, but if cellular elements were present growth followed with or without "Prontosil," and in the former case the cocci were fully virulent. Another assistant found the drug did not affect the growth *in vitro* of ameba. The results in their animal tests led to the theory that the drug might act on the microbe by making it more easily destroyed. This took place, they believed, through the drug's action in preventing the changes associated with adaptation to the animal body such as capsule production. It may be significant that they used in most of their experiments an animal strain of streptococcus, and since such animal strains form capsules much more often than do human strains, the presence of easily demonstrable capsules may have overimpressed them. The theory has to do with adaptive changes which make the bacteria less readily phagocytable, and should be so considered. The writer has not seen any report in which their experiment proving this theory has been repeated. They showed that streptococci previously grown in the animal body *and not in culture media* when injected into other mice were practically unaffected by "Prontosil" therapy. In an earlier article[92b] they gave some evidence that these drugs had effects against leukocidin and hemolysin but they did not develop this evidence in their more complete paper. They[92e] further noted degenerative changes in the extracellular meningococci in treated mice preceding the final sterilization of the exudate. Fourneau[50b] showed that sulphanilamide gave a definite inhibitory effect on molds and certain higher vegetable cells and contrasted this with its innocuous effect on animal cells. Domagk, in a later report,[45c] gave a table of *in vitro* tests with the two "Prontosils" showing no effect on the growth of streptococci, and added that sulphanilamide behaved similarly. He partly agreed with Levaditi's view but believed there were also other factors involved. He considered that in the first phase the drug is bound in the body to the bacteria and he noted swelling and other degenerative changes in the non-phagocyted cocci. Colebrook and Kenny[36a] did not believe the cocci are killed by "Prontosil Soluble" or by a compound formed from it in the body. They found a 2.5% solution killed streptococci in 1 to 3 hours *in vitro* but serum prevented this. The dilution of serum with the drug caused some inhibition of growth. Because their strain of streptococcus formed capsules in defibrinated blood containing the drug as well as without it they did not support Levaditi's idea. Bloch-Michel[20] found mice infected and cured were not immune to later infections. Colebrook[36b] in a number of ingenious experiments was able to demonstrate *in vitro* a certain inhibitory action by sulphanilamide against relatively small numbers of cocci, and very slight effect with serum of treated humans but not with that of mice or rabbits. Tréfouël[155b] considered, since "Prontosil" had been shown to be effec-

tive against "established infections," and also, since animals respond even after the blood is invaded, that these facts were against Levaditi's idea of adaptive changes being the factor. Another finding of interest was the later deaths of treated mice which were found to be sterile and free from lesions. They also noted the absence of immunity in treated mice and were never able to demonstrate agglutinins for the streptococci. (This might suggest that an antigenic alteration had taken place.) Long and Bliss[94a] agreed with others that dilutions of "Prontosil Soluble" had no effect on the growth of hemolytic streptococci, but with sulphanilamide 1:10,000 a definite reduction in growth was observed even in the presence of 50% serum. This dilution also inhibited alpha and gamma streptococci, Types I and II pneumococci, *Neisseriæ* from the throat, *M. tetragenus* and 2 *Hæmophilus* cultures, but had no effect on staphylococcus, certain Salmonella strains and strains of *B. dysenteriæ*. These inhibitions are only effective in increasing the lag after 2 hours' incubation. They did not confirm Levaditi and Vaisman's observations on "capsules," but believed the cocci are in some way damaged before phagocytosis takes place. Rosenthal[127a] had found in 1934 that formaldehyde sulphoxylate would cure mice infected with a *single* strain of Type I pneumococcus, and that these mice were immune to the same and other strains, but this point is not mentioned in his later work[127b] with sulphanilamide. In a third report,[127c] he demonstrated both bacteriostatic and bactericidal action on pneumococci with sulphanilamide in high dilutions *in vitro* and believed this could explain its therapeutic effect, but there was no such action *in vitro* against streptococci, *S. albus* or *E. coli*. Nitti[117] showed sulphanilamide (1:1000) had a definite bactericidal effect against a small inoculum of streptococci in media which did not occur with compounds in which the sulphonamide radical was in the ortho or meta position. They also recorded a similar effect on 2 strains of pneumococci and on *B. abortus* but no effect on staphylococcus, *B. coli* or *B. typhi murium*. Buttle[31] found sulphanilamide 0.04% delayed growth a little with *S. aërtrycke* and *B. typhosus* but had a greater effect on pneumococci and streptococci. They also reported that the treated and surviving *B. typhosus* mice sometimes showed agglutinins and some immunity, which was not the case in the streptococcus mice. (It would be interesting to know which antigen of the bacillus produced these agglutinins.) Bürgers[29] was unable to demonstrate any bactericidal action by "Prontosil" or "Prontosil Soluble" on various strains of streptococci or pneumococci in the test-tube by any method, nor that a linkage with proteins in blood or serum increased this action either *in vitro* or *in vivo*. He failed to find any effect of these drugs on the hemolysin or virulence of many strains, nor did they affect capsule production by Friedländer's bacillus or Type III pneumococcus. Even after prolonged contact of streptococci with "Prontosil" *in vitro* no lessening of virulence was obtained, and he believed that the dye rapidly left the bacteria by diffusion in the peritoneal fluid of the injected mice. However, he did find evidence that streptococci stained by "Prontosil Soluble" *in vitro* became more permeable to vital staining with dilute crystal violet as compared with streptococci from infected mice after being washed and similarly stained. Staphylococci were not so markedly affected by this procedure and pneumococci not at all. Capsules

could not be demonstrated on the peritoneal streptococci, but to the Reviewer that is not a very important argument against an adaptive change, but his failure to find any differences in phagocytic tests *in vitro* was important. However, he showed that streptococci treated with the drug for some hours, then washed and injected subcutaneously in guinea pigs produced reactions very definitely less than were obtained with the untreated washed cultures, and this was fully confirmed in the histologic studies. Similar studies with staphylococcus were negative. Long and Bliss[94a] could not confirm the finding that the serums of treated cases were bactericidal. They showed that streptococci continued growing in the peritoneal cavity of mice for several hours after injection, and they found no evidence that the chemical killed or injured the cocci since the exudate remained fully virulent whether or not the cultures were positive, the positive cultures were always "mucoid" or "matt," and the capsules on the cocci free in the exudate seemed unaffected. They suggested a metabolic change might make them more susceptible to phagocytosis and are quite definite in their belief that the drug effect is on the streptococci and not on the body cells. Buttle[32] had some evidence using small inocula that the blood of monkeys treated with diaminodiphenylsulphone or sulphanilamide became bactericidal for streptococci. Hawking[74] reported that trypanosomes withstand a 1:400 solution of sulphanilamide for 24 hours at 37° C. Mellon and Bambas[106] were unable to show that sulphanilamide had any effect on the dehydrogenases of Type I pneumococcus. They showed, however, that the spinal fluid of a treated case was markedly bacteriostatic for streptococci. Barron and Jacobs[11] found sulphanilamide slightly inhibited the oxidation of glucose by hemolytic streptococci and the oxidation of glucose and lactate by *B. Friedländer*. "Prontosil" had no such effects. Kenny[85] did some 96 tests with the urine of patients receiving sulphanilamide and found the bactericidal effect varied with the amount of drug in the urine. They tested a variety of strains of *B. coli* and other bacteria from the urinary tract and found that *Str. fæcalis* was the only fully resistant one. Dees and Colston[42] quoted Hill as having found that the urine of patients excreting sulphanilamide had no bactericidal activity against beta hemolytic streptococci. Helmholz[76] found the drug excreted in the urine had a decided bactericidal action, particularly in alkaline urine against urea-splitting organisms such as *Proteus ammoniæ*, as well as *B. coli* and *B. aërogenes*. *Str. fæcalis* was also found fully resistant. Mellon and Shinn[107] confirmed Helmholz's results with a 1:10,000 concentration of the drug in urine and recorded the important observation that if urine is added to the initial media, the diluents and the test media, the bacteriostatic and bactericidal effect on *B. coli* is more marked than if broth alone is used. Mellon had previously found a similar potentiative effect with saline using hemolytic streptococci, in that dilution in saline solution enhanced the bacteriostatic effect of the drug and in the presence of normal human serum this effect was further increased. These results give a correlation between *in vitro* tests and *in vivo* clinical results. Gay and Clark[58] found that sulphanilamide had only a bacteriostatic effect in pleural fluid infected with streptococci, and that it never by itself sterilized it. They noted distinct degenerative changes (variation in size and metachromatic staining) in the streptococci under the influence of the drug in serum, and cultures from treated pleural

exudates showed some increase in "matt" over "mucoid" colonies but only in the primary culture. The virulence was unaffected. Bliss and Long[19b] in a study of this problem concluded that inhibition of growth *in vitro*, and a change in the bacteria permitting their phagocytosis *in vivo*, are the only real direct effects produced by sulphanilamide. They failed to demonstrate in the streptococci from treated mice any alteration in type of colony, virulence or capsule formation. Experiments in mice infected intraperitoneally with *B. welchii* and treated by the drug showed the bacteria to be inhibited, and this reduction in multiplication to a rate at which there cannot be produced enough "leukocidin" or other toxins to inhibit rapid phagocytosis explained, they believed, the curative effects in these as in the other bacterial infections. The evidence from the *B. welchii* experiments is somewhat difficult to evaluate. This organism readily forms capsules in the body. The untreated mice died after a few hours obviously from toxemia. Phagocytosis began almost at once. The drug was given before the inoculation. The periodic study of the peritoneal exudate showed marked phagocytosis in both groups at first. As the number of free bacteria in the untreated mice increased so did the number of those phagocyted. In the treated the numbers of both free and phagocyted bacteria decreased. Capsules are not mentioned but there is no evidence that their production did not play a part in the results recorded. This experiment of peritoneal infection with *B. welchii* is not of course directly comparable to gas gangrene in man in which the inhibition of phagocytic action is manifest. However, this experiment clearly demonstrated the striking bacteriostatic effects of the drug.

It is evident that under certain conditions the growth of bacteria is detrimentally affected by these drugs both *in vitro* and *in vivo*. This is indicated by morphologic changes and by inhibitory and even bactericidal effects, but these effects, although seldom strikingly demonstrable, may nevertheless be of primary importance for the final destruction of the bacteria by the defense mechanisms in the body.*

The Effect of These Drugs on the Body Defenses. It is generally accepted that the body defenses are the important and necessary factors in the final curative effects of this type of therapy, and most of the clinical reports emphasize the danger of relying solely on the action of any of these drugs. However, it is not at all clear what actually are the effects of these compounds on the defensive mechanism. That there may be harmful secondary effects is evident from the discussion already given but whether stimulations or other benefits result from their use is undecided.

Domagk[45a] stressed the part played by the neutrophils and the monocytes, as have many others since. Levaditi and Vaisman[92c] showed that these drugs do not prevent the growth of fibroblasts and other cells in tissue cultures. The inconstancy of their experimental results clearly indicated that they were dealing with individual differences in the sensitivity or resistance in their mice, and that the balance of bodily defense is often unstable so that late deaths in treated mice sometimes occurred. Domagk later[45c] showed that the inhibitory effect on bacterial invasion was important and suggested that the monocytes may be activated. Jaeger's[83] results showed "Prontosil" acted locally and he gave some

* Levaditi and Vaisman showed antiendotoxic effects with certain sulphone and sulphoxide drugs (Table 1).

evidence that it stopped excess exudate, changed the character of the pus, softened the wall of abscesses, had an astringent effect, helped the coagulation of blood ("Prontosil Soluble" did not) and like other azo dyes favored granulation tissue. He cited the rapid decrease in the edema in erysipelas as suggestive of the drying effect of "Prontosil." Tréfouël[155] mentioned the finding of cocci in the blood after treatment, and although these bacteria disappeared under further treatment the animals usually died but showed no streptococcal lesions. Long and Bliss[94] believed that the neutrophils at first and the monocytes later were the factors which finally control the infections. In their experiments the drugs were not given until 8 hours after injection of the streptococci and they recorded that all but 4 of 102 mice had positive blood cultures 12 hours after the intraperitoneal inoculation. This may explain why they had so many late deaths in these treated animals. Mice are very sensitive to streptococcal infections and a number of spontaneous epidemics in laboratory mice have been reported, so that there is just a possibility that some of these late deaths may have followed a reinfection by cage contact. Berger's studies[15] on subcutaneously infected mice led him to consider the effects of the drug on abscess formation. He found very little effect on the primary reaction, a certain degree of limitation and a more rapid evolution of the process but quite definitely a prevention of septicemia. Rosenthal[127b] working with sulphanilamide and pneumococci found a much more marked localization of the infection in the mice in which life was prolonged by the drug and there were fewer cocci found in the blood in these mice than in the untreated controls. Bürgers[29] stressed the increase in cellular defense as an individual factor of first importance, and this was indicated by differences in survival among different races of mice and at different times of the year. He also found that mice dying after treatment often had sterile organs. In rabbits, infections were cured even when treatment was delayed 2 to 3 days but not after the fifth day. Gross and his associates[67] did not find that splenectomy prevented the protective action of sulphanilamide in mice given highly virulent streptococci, and the degree of protection was the same as that obtained with normal mice. They recorded that 2 of the splenectomized mice dying after treatment gave negative cultures at autopsy. Long and Bliss[94c] described the wave of neutrophils which appeared in the treated mice 6 to 15 hours after the streptococci were injected intraperitoneally. Cooper and Gross[39a] found in experimental Type III pneumonia in rats that the treated rats which succumbed had bacteremia and peritonitis less often than the untreated fatal cases. Mellon[108] did not find an indication that phagocytosis is a factor in the mechanism of the therapeutic action of these drugs in mice infected *via* the peritoneum with hemolytic streptococci. In guinea pigs, intradermal infections remained localized and rapidly healed in the treated animals, and the histologic response was found to be qualitatively the same as in those with a fatal outcome. The rapid disappearance of pus in treated cases of acute gonorrhea may be illustrated by the results of Dees and Colston[42] as well as by many others. Hofmann[77] found that "Prontosil Soluble" injected into the muscle of dogs penetrated sterile turpentine abscesses as long as these were developing, but these became impermeable to the dye as soon as they were no longer progressive.

These experiments were done in an attempt to explain the failure of this chemotherapy in established abscesses in man. An interesting observation was that 2 dogs with active abscesses suffered from evident loss of appetite and seemed depressed, but these symptoms rapidly improved when the drug was given. This certainly suggests some general effect. Kreidler[90] reported that 1 of 2 rabbits, after an endodermal injection of pneumococcus and not treated until 48 hours, died 9 days later with the local lesion healed and a negative blood culture at autopsy. Basman and Perley's[12] speculation as to the cause of abscess formation in cases of erysipelas has been mentioned above. It is another example of the localizing effect of these drugs. McKinney and Mellon[104] undertook a study to determine the relative importance of the neutrophils and monocytes in this problem of phagocytosis and were able to show that marked differences in cell response followed the injections of different strains of streptococci (Group A). Some mucoid strains are readily taken up by neutrophils, others seem to need macrophages, but the phagocytosis of virulent strains is conditioned by the previous bacteriostatic action of the drug. Bliss and Long,[19b] chiefly on the basis of their results in *B. welchii* peritonitis in that they did not find any decrease in phagocytosis in the untreated mice up to the time of death (a few hours), did not believe the drug stimulated phagocytosis nor that it protected the cells from the products of the bacteria. The need of the leukocytes in the curative process against streptococci was made evident when these cells had been previously reduced by injection of benzene to below 500 per c.mm. of blood, since such mice could not be protected against streptococci by the drug. Gay and Clark[58] studied the problem by experiments in the pleural cavity of rabbits, using a technique perfected by many years of experience. They believed sulphanilamide is a preventive and not a true curative drug. In empyema induced by injections of virulent streptococci blood stream infection was prevented by the drug, the leukocytic count increased as contrasted to a decrease in the controls, the amount of pleural exudate was markedly less and the growth of the streptococcus was greatly reduced. The first cells to appear in both groups were the neutrophils, but in the treated animals the monocytes increased after 36 hours and by 48 hours were predominant. Monocytes were not stimulated *in vivo* by the drug, either in the presence of sterile exudate or killed streptococci. They were found by histologic studies to be massed after 36 hours in the septa and subserous layers of the pleura and some were seen in active mitosis. This accumulation of monocytes was not so striking at the 48-hour period when the pleural cavities became sterile but appeared in quantity from 4 days onward. The course of events in the treated animals was outlined as a bacteriostatic action of the drug, the outpouring of leukocytes and finally for complete sterilization the coöperation of monocytes derived from the local tissues.

The direct transfer of the results from animal experiments to the explanation of the occurrences in human infections is, of course, not justified. In the experimental studies, the most sensitive animals are chosen and the most virulent strains of bacteria are used. There are no preliminary contacts between germ and tissue as happens in the stages of invasion, and the mobilization of defenses to be effective must be very rapid. There is much evidence scattered through the references

I have cited to indicate that the sulphanilamide type of therapy is most effective in the progressive stages of an infection in which active and continuous tissue reactions are taking place. The drug under such circumstances would seem to add the necessary bacteriostatic effect which often tips the balance in favor of a cure. The combined use of antiserum and drug should theoretically add to the curative effects as it has experimentally and practically in a few cases, since antibody defense is often a necessary factor in cellular defense.

The possible general effects of this group of drugs should be more thoroughly studied. Do they in any way affect the inflammatory reaction? Most of the pathogens grow in the interstitial fluids which, if excessive in unchecked inflammation, may embarrass the nutrition of the local tissue cells. Can these chemicals affect the edema of inflammation as has been suggested? Phagocytic cells have been held responsible on excellent experimental evidence for the transfer of pathogenic bacteria to various parts of the body. Leukocytes, in addition to their well established function in phagocytic destruction of bacteria have also high enzymatic activities which are known to injure tissue cells partly by depriving these cells of a portion of their food and partly by direct more or less toxic effects. Further, leukocytes by their action on serum proteins and other substances may provide more easily available nutrients for certain bacteria despite their injurious effect on ingested organisms. Do these drugs have, perchance, a regulating effect or do they prevent an excessive leukocytosis? The clinical findings suggest that on occasion these cells may be disastrously affected and it has been suspected that this is due to the benzene nucleus. It may be that the drugs still retain some of this effect of benzene in a much milder form. Whether these studies may throw some light on the varying infectious processes characteristic for different groups of bacteria the future will determine. There are innumerable problems still to be solved. Some of these comments are admittedly mere speculations, but until we know more exactly the mode of action of these substances, so remarkably effective in chemotherapy, guarded speculation may be justified.

No conclusions are advisable at the present stage of this complicated study. New compounds are constantly appearing which must needs be evaluated. Excellent experimental and clinical methods of study have been evolved, and the future looks bright for even greater progress in this opening field of chemotherapy of bacterial infections.

While this article was in press there appeared the important report by Dochez and Slanetz (Science, **87**, 142, 1938) on the successful Treatment of Canine Distemper—a virus disease—with sodium sulfanilyl sulfanilate.—EDITOR.

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HYGIENE AND PUBLIC HEALTH

UNDER THE CHARGE OF
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SOME RECENT ADVANCES IN DIPHTHERIOLOGY.

ALTHOUGH the field of diphtheria has been the scene of brilliant and epoch-marking discoveries, such as those of Loeffler[39] who isolated the diphtheria organism in 1883, Roux and Yersin[60] who demonstrated the exotoxin of the organism in 1888, and of v. Behring and Kitasato[4] who developed antitoxin in 1890, the physician of today, called to treat an acute inflammatory condition of the throat, is still confronted with much the same problems that have beset clinicians for many decades, even though diphtheria was clearly defined as a pathologic entity as early as 1821 in the classical memoirs of Brettoneau.[7] The modern doctor finds, in addition, that a case of diphtheria is no longer a purely clinical and scientific problem but may also involve sociologic questions arising from administrative regulations. Confronted with these difficulties, the busy practitioner naturally looks to the research worker, in the field and in the laboratory, for new or improved instruments or methods with which to overcome them.

From a purely clinical and therapeutic standpoint relatively little of a fundamental nature has been added to the armamentarium of the physician in recent years except the familiar concentrated and purified antitoxin and certain improved supportive and palliative measures which are matters chiefly of pharmacy and materia medica.

From a more abstract, but nonetheless practical point of view, a number of advances have been made recently which place in the hands of the physician and laboratory worker improved means of determining the presence of diphtheria bacilli in the throats of patients and healthy persons, of isolating them, of determining their virulence and biologic type, and of immunizing susceptible persons.

Bacteriologic Diagnosis. Of immediate interest to the practitioner are those researches dealing with the most rapid and accurate means of obtaining information as to whether or not the throat of his patient harbors diphtheria bacilli; and, if so, whether they are virulent or avirulent. Unfortunately, this particular field of bacteriologic research offers relatively little of which to boast. The cotton swab, rubbed on a slant of Loeffler's medium which is incubated overnight and examined microscopically next morning, still continues as the standard diagnostic procedure. A more rapid but possibly less accurate means of demonstrating the presence of diphtheria bacilli in the nose or throat has recently come into prominence and consists of a sterile swab, dipped into sterile fluid Loeffler's mixture (or plain serum) and then held over a flame just long enough to coagulate the serum. This is rubbed over the patient's throat or nasal passages in the usual manner and then

inserted into a sterile, plugged tube or vial. Growth of the bacteria from the respiratory passages may occur on the serum at the temperature of the physician's vest pocket (30 to 35° C.) in 4 to 8 hours, after which a smear made directly from the swab may show the diphtheria organisms. Longer incubation at 37° C. is desirable when circumstance permits. The idea was originated by Folger[19] and modified (1934) by Solé[67] and later by Brahdy, Brody, *et al.*[6] Unfortunately, the swabs dry out rather quickly before or after use and the serum is apt to crumble in the patient's nose or throat.

A few staining methods designed to render the metachromatic granules (volutin?) of the organisms more prominent were in vogue some years ago; but due to the fact that volutin, or some similarly staining substance, is common to many cocci and other organisms, most competent workers now recognize that these stains often lead to false diagnoses. The methylene blue stain (which has been greatly improved by modern synthetic chemistry) is today more reliable and uniform than ever, and the staining method of Loeffler is still adhered to by conservative and careful bacteriologists.

Possibly we have only recently come to realize that there is a large number of hitherto undescribed forms which may be assumed by the capricious diphtheria bacillus; but it is doubtful if the older workers failed to see these, whether or not they recognized and described them. The "atypical" forms are practically always accompanied by enough typical organisms so that, in a pure culture, they are easily recognized for what they are. However, Van Volkenburgh and the writer[26] have recently brought forward evidence suggesting that in original throat cultures, especially those from healthy carriers, the diphtheria bacilli may be present in large numbers, yet demonstrable only after isolation in pure culture. The data indicate that a negative smear frequently does not prove the absence even of large numbers of *C. diphtheriæ* and that the organisms may be present only in the recently described coccoid forms[34,68] which are almost impossible of recognition in throat cultures containing staphylococci, streptococci and neisseriæ.

The Virulence Test. With respect to the determination of the virulence of diphtheria organisms, the intracutaneous, pure-culture method of Eagleton and Baxter[18] remains the standard technique in most laboratories. A more economical intracutaneous method, designed by Fraser and Weld,[24] is being given some prominence at present. It is described by Fraser and McNabb in the A. P. H. A. Year Book for 1936–1937 (p. 121). Several pure culture suspensions may be injected into the shaved dorsal skin of a rabbit or guinea-pig in 0.1 cc. amounts. After 4 to 7 hours about 500 units of antitoxin are injected intravenously or intracardially and the same doses of the same suspensions are immediately afterward reinjected in sites near the original ones. The damage done in each *original* injection site during the 4 to 7 hours when the rabbit was unprotected by antitoxin is not repaired by the antitoxin and later develops into a typical lesion. The second injection site remains normal and serves as a specificity control. The saving in animals is the principal advantage of this method, as it is subject to the errors which are inherent in all intracutaneous work. The use of the mixed (or "whole culture") method[20,32] is deprecated today except as an emergency, or purely secondary and confirmatory, measure and

it is now recommended that it be used, if at all, only in connection with known clinical cases. The most reliable virulence test is undoubtedly the classical method consisting of the *subcutaneous* injection of 0.5 to 1 cc. of a heavy saline suspension or broth culture of *one strain* of organism into a normal, 250 to 300 gm. guinea pig and into an antitoxin-protected control. Death of the normal pig, or the appearance of a definite local lesion, and complete absence of any change in the control is evidence of virulence. It might be of interest to note, in passing, that recent studies[3,51,57] on the persistence of virulent organisms in the throats of convalescents show quite conclusively that true diphtheria bacilli tend to retain their virulence indefinitely and that the variation of an organism from toxigenic to non-toxigenic must be an extremely rare occurrence. A few instances of the latter appear in the literature;[12,13,44,70] but they are few indeed and much cited and in some of the evidence one may, perhaps, detect that nemesis of the laboratory bacteriologist—contamination. It is probable that diphtheroids become implanted in the normal throat after the cessation of convalescence and that they have often been mistaken for avirulent diphtheria bacilli derived from those originally present during the pathologic condition.

Improved Methods of Cultivation. Since determinations of virulence, as well as scientific studies, necessitate the isolation of diphtheria bacilli in pure culture, numerous attempts have been made to facilitate isolation by means of selective plating media. Many of these media have also been proposed as substitutes for Loeffler's in primary diagnostic work. In the latter field, however, Loeffler's medium has so far held its own against all competitors, although, for primary diagnosis and general cultural work, a simpler medium consisting of 3 volumes of hens' eggs well mixed with 1 volume of water or normal salt solution, was devised a few years ago by Pai.[52] In the writer's hands, at least, it has been found to be the equal of Loeffler's medium in all respects.[41] Its chief virtue lies in the fact that one can obtain eggs readily at almost any time or place, in any amount; a fact in contrast with the difficulty which is often encountered in obtaining and storing quantities of serum as well as the time and expense of preparing dextrose-infusion-broth with which to mix it.

With regard to the isolation of *C. diphtheriæ* in pure culture, the use of minute amounts of potassium tellurite in blood or serum agar for plating media has been found most useful. The tellurite prevents or retards the growth of many of the saprophytic organisms normally contaminating throat swabbings and, in addition, confers a more or less characteristic appearance and a black color on the diphtheria colonies. Although the value of tellurium salts for such work was recognized as early as 1912 by Conradi and Troch,[10] it first received world-wide notice following the researches of Clauberg,[8a,b] who at first devised a somewhat complex and cumbersome medium containing glycerin, blood and tellurite. Numerous modifications were later made, nearly all of which tended toward simplification. Possibly the simplest and most generally valuable modifications are those devised by McCleod *et al.*,[1] by Douglas,[16] and by the writer.[25] The latter consists merely of 5% blood agar to which is added about 0.04% potassium tellurite and 0.005% cystine; the cystine has been shown by Schmidt[63]

and others to be essential to the growth of the organism because of its sulphydryl group (SH).

Opinion differs as to the advisability of using such selective media in place of Loeffler's or Pai's for primary diagnostic work. Cystine is is known to distort the morphologic appearance of *C. diphtheriæ* so as to render the organisms almost unrecognizable except by the expert while tellurite, being very toxic, may well prevent growth of the diphtheria organisms when the swab contains very few of them to start with, especially since, coming freshly from the throat, they find on tellurite agar at best a somewhat strange and inhospitable environment. This is notably true in dealing with healthy carriers. However, many workers,[1,31,62] especially in Europe, are now using as a routine procedure the inoculation of plates of some variety of tellurite medium directly with throat swabs and basing their diagnosis on the appearance or non-appearance of diphtheria colonies, which are generally characterized by a gun-metal or black color and can be recognized (usually) by one familiar with them. When growth occurs, isolation is of course advanced by 24 hours. It has recently been shown,[26,27] during surveys of school children, that by first inoculating Loeffler's medium and, after 18 hours' incubation, smearing the growth on cystine-tellurite medium, the number of carriers detected may be greatly increased over those found by the mere microscopic examination of the growth on the Loeffler's tubes.

Gravis and Mitis Types. The organisms isolated during the surveys referred to above were found to be rather uniform in their cultural characters, but this has not been the experience elsewhere, and biochemical differences between strains of *C. diphtheriæ* isolated from different types of cases have been the subject of world-wide discussion for several years. In 1931, Anderson, Happold, *et al.*[1] described two types of *C. diphtheriæ* which they called, respectively, *C. diphtheriæ gravis* and *C. diphtheriæ mitis*. The former was said to be characterized by its fermentation of starch and glycogen; the formation (on McLeod's "chocolate" tellurite medium) of a distinctive type of rough colony; a peculiar, diphtheroid-like morphology; absence of hemolytic power; ability to form pellicles on broth, and a rapid alkalinization of broth. The mitis types had characters generally the opposite or negative in these respects, fermenting neither glycogen nor starch, producing smooth colonies, a typical morphology, no pellicles, demonstrable amounts of hemolysin and slow alkalinization of broth. *C. diphtheriæ gravis* was found associated with severe, fulminating and often fatal cases, while the mitis types were usually found in mild cases and in healthy carriers, and were frequently avirulent. A type spoken of as "intermediate" was described on the basis of only 6 cultures and resembled the mitis type except for a slight flatness in colony form and a granular growth in broth, both of which are trivial differential characteristics. A great deal of work was done following the original report of Anderson *et al.* The reader interested in detail is referred to the list of references appended hereto.[11,45,46,54,58,61,72]

As is all too frequently the case, subsequent workers have disagreed with the original reports of Anderson *et al.*, and also among themselves. One very obvious reason for the diversity of opinions in this instance is that each worker has set up his own definition of the *gravis* and *mitis*

types and it is impossible to compare any two sets of data so far reported in the literature on exactly the same basis. From the welter of confusing evidence, however, and judging by the writer's own experience, the conclusion seems indicated that gravis-like and mitis-like types are encountered, but that all of their distinctive characters are variable, that they often tend to vary toward the opposite type with regard to any cultural character, both *in vitro* and in nature, and that no one or more biochemical characteristics (including starch fermentation) are necessarily associated with the clinical severity of any case of diphtheria. Starch fermentation, a widely used differential test, is especially subject to experimental error. Further, the determination of type is of no clinical or prognostic value because of the time required. Studies of type do seem, however, to have a distinct usefulness in following the epidemiology of diphtheria.

The toxins of the various types seem to be qualitatively identical,[46,54,55] although strangely enough the gravis strains are sometimes poor toxin producers (*in vitro*) as compared with mitis.[42,43] However, it is possible that certain strains, such as the gravis type, may possess enhanced powers of maintaining themselves in the throat in spite of antitoxin therapy or that, in the human throat, certain toxic substances are produced which do not appear in artificial culture media.

Active Immunization. With regard to active immunization against diphtheria some notable achievements of recent years may be cited. Shortly after the work of Behring and Kitasato (1890) (probably at the suggestion of Theobald Smith) it became the general practice to use, for the immunization of children against diphtheria, small amounts of diphtheria toxin which had been *almost* completely neutralized with antitoxin. Three injections of 1 cc. of such mixtures, given at weekly intervals, were required. The method was effective but cumbersome and there were some accidents resulting from improperly neutralized toxin and from a dissociation of the toxin and the antitoxin due to freezing. However, "T. A. T.," as the immunizing mixture was familiarly called, was for years widely and successfully used.

A distinct advance in the fight against diphtheria was made in 1921 when it was observed by Glenny and Sudmerson[29] that diphtheria toxin which had lost its toxicity with the passage of time, or as a result of the action of various agents (such as heat, light, or chemicals, especially *formaldehyde*) was still just as *antigenic* as the toxin from which the detoxified product (toxoid) had been prepared. The advantages in using such material as an immunizing agent in the control of diphtheria.[56b] were realized by everyone in the field; but the name of Ramon in France is prominently associated with the exploitation of formalin-toxoid or, as he called it, "anatoxin." Potent, toxin-containing broth is treated with about 0.4 % of formalin at 37° C. for 4 to 6 weeks. The material is then tested for loss of toxicity and for antigenic power and is sold as toxoid. The formalin is believed to combine with some part of the toxin molecule so that toxicity is lost but specific antigenic properties are retained. Whatever the explanation may be, many scientists confirmed the work of Glenny, Ramon, and their colleagues. Ramon[56a] also showed that the formalin toxoid could be flocculated promptly and quantitatively by an exactly neutralizing quantity of antitoxin, for example, 1 unit (hence the "L f dose" or "flocculating

unit" of toxoid), and it could, like toxoid, be standardized by this means. Two or three 1 cc. (12 to 15 L f) doses of the formalin-toxoid were found by many[15,23,37] to be as effective in immunizing as the T. A. T. and yet obviating the dangers inherent in the latter.

One difficulty with toxoid was (and is) that disagreeable reactions occur in many children of about 7 years of age, or over. These are believed by many to be of an allergic nature due to the proteins of the meat, peptone, or bacterial cells used in preparing the cultures. Others ascribe them to the toxoid itself. Probably both are involved. Therefore attempts were made to remove the extraneous substances from toxoid broth by flocculation and precipitation methods, applying the knowledge gained as early as 1902 by Danysz[14] that various precipitates adsorb toxoid. Antitoxin, as a flocculating agent, was known to be effective, but, of course, introduced still more foreign protein. However, it was observed by Glenny and Barr[28] that the precipitate which potassium alum formed upon its addition to toxoid-containing broth, adsorbed and held all the toxoid. Wells, Graham, and Havens[71] and later Havens and Wells[33] purified such alum-precipitated toxoid by removing the supernatant fluid, washing the precipitate in saline solution and then resuspending it in a volume of saline solution equal to the original toxoid. The milky fluid thus obtained was found to contain practically all of the original toxoid and was said to be a more effective immunizing agent than the anatoxin of Ramon. Evidence has since been brought forward[2,40] indicating that 1 dose of this alum-precipitated toxoid has the same immunizing value as 3 doses of T. A. T. or of unprecipitated toxoid, and in some fields it has come to supplant the latter two. However, it is still subject to the same objections as the old formol-toxoid; *i. e.*, the frequent occurrence of sometimes severe (but usually local) reactions in older children. The alum itself may have some part in this effect. On the other hand, there are competent authorities[22] who hold the anatoxin to be the superior antigen, and indeed the matter needs further elucidation.

One advance of some importance resulted from various studies of the allergic reactions referred to above. It has been customary to use, as a control in the Schick test, exactly the same material as is used for the test itself, but heated at about 80° C. for 15 to 30 minutes. Moloney and Fraser[49] tried formol-toxoid as a control in the Schick test. They observed numerous pseudoreactions but found them to occur chiefly in persons who were likely to react severely against immunizing doses of toxoid. By the use of a preliminary intradermal injection of a *suitable dilution of a selected toxoid*, therefore, it was found possible to avoid severe reactions in persons sensitive to toxoid. The "Moloney test," as it is called, is still under investigation, both as a control for the Schick reaction and as a guide in toxoid immunization.[48,69]

The immunizing efficacy claimed for alum-precipitated toxoid is ascribed to a continuous and *prolonged* stimulation of antibodies due to slow absorption of antigen, by the tissues, from the surface of the particles of alum precipitate in the locus of the injection. The original fluid toxoid of Ramon is very quickly absorbed and excreted and its stimulating action, being transitory, has to be applied repeatedly in the form of 2 or 3 successive doses.

This rapid absorption of the soluble material may have been the principal reason for unsatisfactory results at first obtained with very

pure and highly concentrated (50 to 70 L f per cc.) toxoids developed by Schmidt and his collaborators,[65,66] using aluminum hydroxide as the precipitant and then redissolving the toxoid in small volumes of Na_2HPO_4 solution. The rate of absorption of this fluid product was later retarded by mixing a small amount of $Al(OH_3)$ with the purified, concentrated toxoid.[64] Leach, Jensen,[38] and others report very satisfactory results with the latter antigen; a single dose of 1 or 2 cc. (35 to 50 L f per cc.) producing immunization in a large percentage of children tested, with minimal untoward reactions.

Coincident with these studies Jensen and others, using a modification[21,35] of the intracutaneous serum-antitoxin-titration method originated by Römer,[59] have reinvestigated the relation between the Schick reaction and the serum antitoxin titer, the rate of immunization, and the rate, duration and decline of immunity of individuals and populations of different age composition, sex, color and previous natural and artificial contact with diphtheria bacilli, toxin or toxoids. Many other studies bearing on the problems of diphtheria control have also been possible by this means, and a great number of interesting and important facts have thus been brought to light. For example, Michiels and Schick,[47] investigating the relationship between the Schick reaction and the antitoxin titer in human serum, had earlier arrived at $\frac{1}{30}$ to $\frac{1}{50}$ unit per cc. of patient's serum as the quantity of antitoxin necessary to give a negative Schick test. Persons whose serum contained less than $\frac{1}{30}$ to $\frac{1}{50}$ unit per cc. generally gave a positive Schick reaction. More accurate studies of v. Gröer and Kassowitz,[30] recently confirmed by Fraser, Jensen, and others have since shown that $\frac{1}{2}\frac{1}{50}$ unit is more nearly the level at which the Schick test changes from positive to negative. It seems likely that the later workers used purer toxin, which had less of the impurities tending to interfere with, or non-specifically to combine with, the serum antitoxin of the patient.

Black,[5] in 1934, published data suggesting that colored children respond more quickly to the antigenic stimulus of T. A. T., and therefore, inferentially, of toxoid and of actual infection, than white children. Further, individuals of either race have been found to show marked personal differences in immunizability or rate of response to diphtheria antigens. This is of importance epidemiologically and clinically. Obviously, a child whose body responds by the production of antitoxin a few hours after diphtheria bacilli have lodged in its throat is less likely to contract clinical diphtheria than children who respond slowly or not at all.

The effect of a primary and a secondary stimulus on the rate of antitoxin production by human beings has recently been under investigation and an interesting and promising method, not only of inducing but also of *maintaining* immunity in a population, even including the immunologically refractory individuals, and involving only a single subcutaneous injection of toxoid, has been brought forward by Jensen.[35]

The method is based on an immunologic phenomenon originally observed by Cole in 1904,[9] who studied the immune reactions of rabbits being injected with typhoid bacilli. In animals whose tissues had once been in effective contact with typhoid bacilli but whose agglutinin titer had fallen very low with the passage of time, it was found that the original high titer quickly (in a few hours) returned following a single small injection of the specific antigen. The phenomenon seems to be

of broad, fundamental significance and its occurrence is probably to be observed in connection with many other antigens. In any event it has been observed in the case of diphtheria toxin; so small a stimulus as the Schick[17] test having a prompt and well-marked effect on the antitoxin titer of the blood serum, especially in persons whose tissues have already had some experience (primary stimulus) with diphtheria bacilli or their toxic products.

The method of immunization proposed by Jensen is based on these observations and consists of a primary injection of 1 to 1.5 cc. (≡35 to 50 flocculating units) of Schmidt's purified and concentrated toxoid mixed with 10 volumes % of a special $Al(OH_3)$ suspension. This constitutes the "primary stimulus." It is followed after an interval of 4 weeks, by 3 to 6 *intranasal* instillations, of 25 flocculating units each, of the clear $Al(OH_3)$-free concentrate, given about 2 to 7 days apart. These constitute the "secondary stimulus." The immunity response has been found highly satisfactory in all cases tested; disagreeable reactions to the first injection being relatively slight, and those to the subsequent nasal instillations *nil*. The possibility of leaving the instillations to the mother after the first injections are made, is pointed out. There are obviously many aspects of this problem which remain to be worked out in detail. Recognition of the fact that a primary stimulus prepares the tissues so that they respond promptly to any ordinary infection is, however, an important advance. The relative rarity of diphtheria in adults, even though many are known to be Schick positive, is probably dependent on their ability to mobilize their defenses almost instantly upon invasion by a toxigenic organism.

New Media for Toxin Production. We may close this survey with a brief paragraph concerning the researches of Mueller,[50] Pappenheimer[53] and their associates, on the metabolic requirements of *C. diphtheriæ*, which have culminated in one of the most fundamental and suggestive discoveries made in recent years. One of the great obstacles to an exact knowledge of the growth and functioning of bacteria and their toxins, both in the human body and in the test tube, has been the fact that as far as was known the organisms would grow and produce toxin only in broth containing such empirical and variable mixtures as peptone and meat infusion. After a long series of experiments, a medium was finally prepared consisting *only* of pure water and pure chemical compounds of *known* formula and origin, such as glycine, cystine, pimelic acid, $MgSO_4$, nicotinic acid, NaCl, and so forth; a synthetic medium in brief, in which diphtheria bacilli will grow abundantly and produce relatively enormous quantities of toxin. The value of such a discovery is immediately apparent not only in diphtheriology but in other branches of bacteriology. It seems very likely that in the near future the present methods of preparing antigens for the prevention of diphtheria and other diseases will become obsolete as a result of this work. Only time can reveal the greatest significance of such studies as these.

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PHYSIOLOGY

PROCEEDINGS OF
THE PHYSIOLOGICAL SOCIETY OF PHILADELPHIA
SESSION OF JANUARY 17, 1938

The Action of Sodium Bisulphite and Sulphanilamide on Purine and Pyrimidine Compounds With the Production of Hemolysins, and a Suggested Mechanism of the Action of Sulphanilamide on Hemolytic Streptococci. E. J. CZARNETZKY and M. G. SEVAG (Department of Bacteriology, School of Medicine, University of Pennsylvania). Hope of finding a chemical entity capable of destroying bacteria specifically without injury to the host seems to have been realized in the discovery of the action of sulphanilamide on streptococci. In order to take advantage of this finding in a search for new specifics which would combat other types of bacterial infections, it is necessary to determine the basic chemical reactions underlying the bacterial action of sulphanilamide.

There is good evidence for the belief that the surface of β-hemolytic streptococci of Lancefield's Group A contains an antigen which is partly composed of nucleic acid. This antigen can be isolated in a purified form, and when treated with sodium bisulphite or sulphanilamide it becomes lytic for red blood cells *in vitro*. Yeast nucleic acid and vitamin B_1 are made hemolytically active in the same manner by both reagents. It is known that the reaction between vitamin B_1 and sodium bisulphite results in the formation of a sulphonic acid derivative of the pyrimidine ring of the vitamin. Such a sulphonic acid derivative is hemolytic. Whether a similar chemical reaction takes place between sodium bisulphite and yeast nucleic acid or streptococcal nucleic acid, resulting in the production of hemolysins, has as yet not been verified.

A consideration of the toxic effects of sulphanilamide which include hypochromic anemia and granulopenia seems to be in harmony with the fact that sulphanilamide may react with nucleic acid compounds with a resultant formation of hemolysins.

The possibility that inorganic sulphites might be substituted for sulphanilamide in the chemotherapy of streptococcal infections is being investigated.

The Use of Lyophile Plasma in Correcting Hypoproteinemia and Preventing Wound Disruption. W. D. THOMPSON, JR., I. S. RAVDIN, and I. L. FRANK (Laboratory of Surgical Research, University of Pennsylvania). In previous experiments in hypoproteinemic dogs we observed a high incidence of abdominal wound disruption after laparotomy. The present experiments were devised to determine whether wound disruption in hypoproteinemic dogs could be prevented if the hypoproteinemia was corrected by the intravenous administration of serum after laparotomy. In such animals, the serum protein level was normal within 7 days following operation. Fibroblastic proliferation progressed at a normal rate in these animals and complete repair was present within 14 days.

The Effects of Pressor Drugs and of Saline Kidney Extracts on Blood Pressure and Skin Temperature of the Rabbit's Ear. EUGENE M. LANDIS, HUGH MONTGOMERY, and DONALD SPARKMAN (Departments of Pharmacology and Medicine, University of Pennsylvania). Many pressor substances have been studied to determine their effects on blood pressure, relatively little attention being paid to their action on the peripheral blood-vessels. It has been shown recently (Prinzmetal and Wilson, Pickering) that in human hypertension abnormally high blood pressure is associated with normal peripheral blood flow, even when vasoconstrictor tone in the extremities is diminished by warming the body. It seemed highly desirable, therefore, to determine whether the action of known pressor substances simulates human hypertension to the extent of producing, in an experimental animal, a rise of blood pressure without reducing peripheral blood flow simultaneously.

The usual oscillometric technique was modified to permit photographic recording of systolic blood pressure and pulse amplitude in the central artery of the rabbit's ear while vasoconstrictor tone of nervous origin was abolished by warming the body of the rabbit. Changes of peripheral blood flow were determined by measuring skin temperature. This procedure (1) did not require anesthesia, (2) excluded spontaneous variations in vasoconstrictor tone, (3) avoided trauma of the vessels studied, (4) permitted repeated observations on the same animal over periods of days, and (5) allowed slow intravenous injection of drugs or extracts at a constant rate over long periods of time.

Adrenalin, tyramine, pituitrin, pitressin, ergotoxine, ergotamine, guanidine, methylguanidine and dimethylguanidine all elevated blood pressure to some degree in sufficient dosage, but simultaneously constricted the auricular vessels and always diminished peripheral blood flow. Unheated 5% saline extracts of normal rabbit kidney usually reduced, and only occasionally elevated, blood pressure; peripheral blood flow, however, always decreased during the pressor response.

Similar 5% saline extracts of normal rabbit kidney, treated by heating to between 55° and 56° C., followed by filtration at or near 55° C., had no depressor action but always raised blood pressure conspicuously without decreasing skin temperature during any part of the pressor response. This property of elevating blood pressure without reducing peripheral blood flow depended upon a protein-like substance which could not pass an ultrafilter, remained in solution up to 56° C., was precipitated or destroyed by heating to 65° C., and could be precipitated by ammonium sulphate, resembling in these respects the substance named "renin" by Tigerstedt and Bergmann.

Of all the pressor substances studied, these specially heated kidney extracts, and the protein-like material precipitated from such extracts, were the only ones which elevated blood pressure without decreasing peripheral blood flow, a relationship which is also characteristic of certain forms of human hypertension.

The Action of Chemical Substances on the Cerebral Circulation. C. F. SCHMIDT and J. P. HENDRIX (Laboratory of Pharmacology, University of Pennsylvania). In anesthetized cats intracranial (parietal

cortex) and extracranial (tongue or mylohyoid muscle) blood flow was measured simultaneously by thermocouples. Drugs were injected into the ipsilateral common carotid.

Cutting the ipsilateral cervical sympathetic nerve usually increased extracranial blood flow, never intracranial flow. Weak stimulation of the cephalic end always reduced extracranial flow, seldom intracranial flow. *Vasodilators* varied greatly in their relative effectiveness on intracranial and extracranial blood flow: CO_2 (inhaled) increased intracranial and decreased extracranial, histamine increased extracranial and decreased intracranial, nitroglycerine increased both about equally, while cholines increased extracranial markedly, intracranial slightly, and caffeine was relatively ineffective on both; ether (inhaled) increased intracranial flow and decreased extracranial until vasomotor depression occurred. *Vasoconstrictors* (adrenalin, ephedrine, pituitrin, pitressin) decreased extracranial flow markedly, intracranial not at all. Only ergotamine showed an inconstant constrictor action upon intracranial vessels, and this only in large dosage.

In rabbits, intracranial blood flow was restricted to one internal carotid, by ligating the opposite internal carotid and the basilar arteries, and measured by a thermostromuhr on the common carotid. Simultaneously flow was measured in the parietal cortex by a thermocouple. Stimulation of the ipsilateral cervical sympathetic usually reduced total and parietal flows, the latter somewhat less. Occasionally reduction was sufficient to produce symptoms of cerebral anemia. In one experiment, fatal spasm of cerebral vessels resulted, relieved temporarily by nitroglycerine. CO_2 increased total and parietal flow; quantitative measurements indicate that the increase in total flow may exceed 100%. The effects of drugs were essentially the same on both flows and similar to those seen in cats.

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